Toxicology and Applied Pharmacology 338 (2018) 9–19

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/taap

Temporal cytokine and lymphoid responses to an inhaled TLR7 antedrug agonist in the cynomolgus monkey demonstrates potential safety and tolerability of this approach

John Bell[a], Mike Dymond[b], Mark Biffen[a], Stephen Delaney[a], David Keeling[a], Hui Zhang[c], Ian Robinson[d,*]

[a] Respiratory, Inflammation and Autoimmunity, IMED Biotech Unit, AstraZeneca, Gothenburg, Sweden
[b] Plus-Project Ltd, Alderley Park, Cheshire, United Kingdom
[c] Drug Safety and Metabolism, IMED Biotech Unit, AstraZeneca, Gothenburg, Sweden
[d] Drug Safety and Metabolism, IMED Biotech Unit, AstraZeneca, Cambridge, United Kingdom

ARTICLE INFO

Keywords:
TLR7
AZD8848
Asthma
Cynomolgus
Safety assessment
Antedrug

ABSTRACT

AZD8848 is a TLR7 agonist antedrug developed for administration by inhalation dosing for the treatment of allergic diseases, such as asthma. Allergic asthma is associated with increased levels of Th2 cytokines which are suppressed for extended periods by TLR7 agonists in a number of preclinical models of allergic airway inflammation. However, TLRs form a central part of innate immunity and their activation often results in proinflammatory responses. Whilst AZD8848's antedrug mechanism is designed to restrict its pharmacological action beyond the lung, the effect of chronic, supramaximal dosing to the target tissue has yet to be defined. To support clinical development of this potentially disease modifying approach the nonclinical safety and pharmacodynamics of AZD8848 were evaluated in cynomolgus monkeys in studies examining single or multiple weekly inhaled doses. Here we show that following a single dose nearly all responses returned to baseline within a week. During multiple dosing serum biomarkers were quantified over the dosing period and indicated a limited systemic response. The dose at which maximal interferon responses were seen was dependent on dose. Thorough histopathological examination revealed a dose related increase of size and cells of lymphoid tissues in the lung and nose. Local lymphoid responses were recovered after the treatment free period. These studies are the first to evaluate safety of an inhaled TLR7 agonist and demonstrate AZD8848 is safe with a no observed adverse effect level at 26 μg/kg allowing progression to man with weekly inhalation dosing.

1. Introduction

Asthma is a disease that is still increasing in prevalence and where there remains considerable unmet clinical need (Pawankar, 2014; Pearce et al., 2007). We have been investigating the possibility of developing a novel, disease-modifying treatment for asthma through the selective activation of TLR7. Asthma is commonly associated with an inflammatory Th2 imbalance with elevated levels of cytokines such as IL-4, IL-5 and IL-13. Numerous studies have shown in vitro and in vivo that TLR7 agonists suppress the production of Th2 cytokines and reverse changes associated with the Th2 phenotype in animal models (Camateros et al., 2007; Matsui et al., 2012; Vultaggio et al., 2009; Xirakia et al., 2010). The mechanism by which this occurs is believed to be through the generation of Type-1 interferons (IFNs) following TLR7 activation (Matsui et al., 2012). TLR7 agonists have been tested in man for a number of disease indications including cancer and cytomegalovirus infection. However, they have been associated with side effects, such as influenza-like symptoms, linked to the systemic production of Type I IFNs (Dudek et al., 2007; Fidock et al., 2011; Pockros et al., 2007). For the treatment of asthma, there is an opportunity to deliver drug directly to the lung and avoid unwanted systemic effects. Working in collaboration with Sumitomo Dainippon Pharma, we have developed a series of TLR7 agonist antedrugs, including AZD8848, whose plasma esterase lability is designed to prevent systemic exposure (Kurimoto et al., 2010). This is, to our knowledge, the first and only TLR antedrug that has been developed as a potential therapeutic molecule and the

Abbreviations: BALF, bronchoalveolar lavage fluid; BALT, bronchus-associated lymphoid tissue; CRP, C reactive protein; MALT, mucosa-associated lymphoid tissue; NALT, nose-associated lymphoid tissue

* Corresponding author.
E-mail address: Ian.robinson@astrazeneca.com (I. Robinson).

https://doi.org/10.1016/j.taap.2017.11.002
Received 6 July 2017; Received in revised form 30 October 2017; Accepted 3 November 2017
Available online 06 November 2017
0041-008X/

first designed to be delivered by inhalation. Following inhalation delivery to the nose or the lung, these compounds achieve sufficient concentration to activate TLR7 locally, but do not achieve plasma concentrations sufficient to activate TLR7 systemically either in pre-clinical models or in man (Biffen et al., 2012; Delaney et al., 2016; Greiff et al., 2012, 2015). Nevertheless, this antedrug approach remains susceptible to locally generated IFNs, or other cytokines, spilling over from the lung to the systemic circulation.

In pre-clinical models of allergic asthma, TLR7 agonist antedrugs successfully inhibited the allergen-induced increase in bronchoalveolar eosinophils and Th2 cytokine production (Delaney et al., 2016; Matsui et al., 2012). Despite the short term exposure of these antedrug compounds, further studies showed that once-weekly dosing over 6–8 weeks successfully induced a suppression of the Th2 responses which remained for a period of up to 3 months following cessation of dosing (Aoki et al., 2010; Delaney et al., 2016; Matsui et al., 2012). Should such an effect translate to man, it could open the way to a disease-modifying approach to asthma whereby short periods of treatment would allow long periods of asthma control in the absence of further medication. When administered directly to the nose in clinical studies AZD8848, given once weekly for five to eight weeks, was shown to reduce allergen-induced symptoms of allergic rhinitis (Greiff et al., 2012, 2015) and allergen-induced bronchoconstriction in asthmatics (Leaker et al., 2012) up to one week following the end of dosing. However, as this benefit was no longer evident four weeks after dosing, we reasoned that to maximize the effect of AZD8848 and emulate the long duration of action seen in pre-clinical models, administration directly to the lung might be required.

As stimulation of the innate immune response with a TLR7 agonist has the potential to influence and reprogramme the adaptive immune response we wanted to assess the pharmacodynamic and potential toxicological effect of single and multiple inhaled doses of the antedrug AZD8848 in a suitable animal species. In order to model the antedrug concept a species was needed in which the plasma lability of AZD8848 closely matched that of human. This was the case for cynomolgus monkey, but not for any other standard large animal toxicological species investigated, including dog and mini-pig. Furthermore data for non-human primates shows a similar cellular pattern of expression of TLRs compared to man (Ketloy et al., 2008) leading to the choice of cynomolgus monkeys for these studies. Previous in vivo rodent studies had indicated that once weekly dosing of AZD8848 was able to achieve an inhibition of allergic responses up to 26 days after the cessation of dosing (Delaney et al., 2016). No advantage of more frequent dosing was demonstrated in the intranasal administration of AZD8848 in early clinical studies in allergic rhinitis where adverse events were more frequently observed during 3 weekly intra-nasal doses of 20 μg of AZD8848 compared to once weekly dosing of 60 μg (Greiff et al., 2015). To understand the pharmacodynamic profile resulting from weekly inhalation of a TLR7 agonist antedrug we studied the duration of changes to a range of cytokines both locally from the lung and systemically. Having identified neopterin as a mediator that was elevated following inhalation of AZD8848, this and the acute phase inflammatory markers CRP and haptoglobin were monitored to assess both changes in the pharmacodynamic effect of TLR7 agonism and any systemic pro-inflammatory response following repeated weekly dosing. In this study we also carried out a full safety evaluation of AZD8848 following repeated weekly dosing to determine tolerable limits and a suitable clinical starting dose before progressing to man. This study enabled us to understand the local and systemic cytokine signature following inhalation dosing with a TLR7 antedrug agonist and identified levels which were safe and tolerated in preparation for further studies in man.

2. Methods

2.1. Test compound and formulation

The test compound, AZD8848, was synthesized at Sumitomo Dainippon Pharma and was administered as a pure micronized dry powder aerosol generated using a Wright Dust Feed (WDF) mechanism (small canister, packed at 5 bar pressure and operated at 20 L/min dry air flow). Doses were administered simultaneously to all animals in each dose group by passive inhalation via a conditioning/dilution chamber to individual face masks. Different doses of AZD8848 were achieved by varying the concentrations of the compound in the exposure system (by adjusting the WDF operating speed and, for lower dose groups, by dilution of the generated aerosol with dry air) whilst keeping the duration of exposure constant (10 min). Control treatment in the pharmacokinetic/pharmacodynamic study comprised a dose of inhaled micronized lactose, whereas in the toxicology studies, control animals were given air only. Actual aerosol concentrations delivered to the animals (mean of 0.23, 1.1, 6.1, 13, 51 and 238 μg/L for the toxicology studies and 0.22, 1.1, 5.5 and 54 μg/L for the PK/PD study) were determined by UPLC analysis of aerosol samples collected on open face quartz fibre filters. Measurement of aerosol particle size distribution showed mass median aerodynamic diameters (MMADs) of 2.7 μm for lactose and 1.0–1.7 μm for AZD8848, with geometric standard deviations (GSDs) of 2.3 and 1.7–2.1, confirming all aerosols to be highly respirable.

The calculated inhaled dose was derived from the following equation:

$$\frac{\text{Analytical aerosol concentration of AZD8848 (μg/L)} \times \text{Volume of air inhaled in 10 min (L)}}{\text{Bodyweight (kg)}}$$

2.2. In vitro assays

Activity against human or cynomolgus TLR7 in reporter cells, the preparation of PBMCs and methodology for the proliferation assay as well as in vitro plasma stability determinations were performed as described previously (Biffen et al., 2012). Briefly, in reporter assays, HEK293 cells stably transfected with human or cynomolgus TLR7 and pNiFty2-SEAP reporter constructs curves were seeded into flat bottom polystyrene 96 v-well plates and dose-responses were generated by addition of test compounds and incubation for 20 h.

For PBMC assays blood from cynomolgus monkeys was collected into 9 mL Lithium (Li)-heparin tubes. To isolate the PBMCs the blood from each Li-heparin tube was centrifuged at 830 g at room temperature for 10 min and the plasma removed. The blood pellet in each tube was resuspended in 5 mL Hanks' Balanced Salts Solution (HBSS) and layered onto 5 mL lymphoprep and centrifuged at 1090*g* for 20 min. The PBMC layer was removed and washed twice with HBSS, recovering the cell pellet by centrifuging at 400*g* for 10 min. The PBMCs were resuspended in assay medium and counted either with a haemocytometer or by Coulter Counter and diluted to 1.1×10^6 cells/mL in assay medium. The proliferation assay was performed in 96 well clear flat-bottomed tissue culture plates. 20 μL test compound (prepared as 10 × stocks described above) or 20 μL assay medium (RPMI 1640 with 25 mM HEPES, 10% FBS, 2 mM L-glutamine, 10 U/mL penicillin, 10 μg/mL streptomycin) containing 1% DMSO was added per well followed by 180 μL PBMCs (200,000) prepared above. PBMCs and compound were incubated for approximately 44 h at 37 °C in an atmosphere of air/CO_2 (95/5 v/v) before addition of 0.0185 MBq (0.5 μCi) [^{3}H]-Thymidine in 10 μL medium. Following a further 6 h incubation the cells were filtered onto glass fibre filter mats and washed extensively in water using a Tomtec filtration apparatus and filter bound radioactivity was quantified with a MicroBeta 1450 Trilux (Perkin Elmer).