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Pharmacokinetics and sputum penetration of azithromycin during once weekly dosing in cystic fibrosis patients

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Abstract

In this study we examined pharmacokinetics, systemic exposure and sputum penetration of azithromycin (AZM) in CF patients on chronic daily AZM therapy after changing to a once weekly dosing scheme.

Eight adult CF patients using AZM 500 mg/day were changed to a once weekly dose of 1000 mg during 3 months. Once per month sputum and blood samples were collected. AZM was quantified in blood plasma and polymorphonuclear neutrophils.

The cumulative weekly dose was reduced with a factor of 3.5 (7×500 mg vs. 1×1000 mg weekly).This led to a reduction in area under the curve (AUC ± S.D.) with a factor of 2.5 ± 0.8 in plasma, 2.8 ± 0.9 in blood, 2.2 ± 1.1 in PMNNs and to a reduction in average sputum concentration with a factor of 3.0 (± 1.5).

At 1000 mg once weekly reduced but still substantial concentrations were achieved in PMNNs and in sputum. Although not significant, a tendency towards less than linear reduction was found. In order to calculate and propose an optimal dosing scheme we need to establish a relation between exposure levels and clinical efficacy.

Keywords: Azithromycin; Pharmacokinetics; Sputum; Penetration; Polymorphonuclear neutrophil; Once weekly dosing

1. Introduction

Chronic use of azithromycin (AZM) reduces or stabilizes clinical symptoms of airway inflammation associated with chronic infection of *Pseudomonas aeruginosa* in patients with cystic fibrosis (CF). Clinical studies demonstrating this effect have been performed with different dosing schedules ranging from 250 mg to 500 mg 3 times per week to 250 mg once daily). All randomized controlled trials comparing AZM with placebo have shown a significant advantage in ΔFEV_1 and a reduction in number of exacerbations in patients on AZM [1–6].

We have previously reported pharmacokinetic data for AZM after chronic administration of 500 mg daily. AZM was quantified in plasma, blood, isolated polymorphonuclear neutrophils (PMNNs) and sputum of 8 adult CF patients and demonstrated an extended elimination $t_{1/2}$ in plasma (102 ± 20 h), blood (180 ± 68 h) and in PMNNs (289 ± 166 h) [7]. In blood we found a C_{max} of 2.01 ± 0.74 mg/l at T_{max} of 3 ± 1.1 h of which 1.44 ± 0.69 mg/l (72%) was found in PMNNs. In sputum the concentration was between 12 and 53 mg/l (immediately after the last dose) and was still between 4 and 27 mg/l 10 days after the last dose. On average, the concentration we found in PMNNs was 2100 times the C_{plasma} 24 h after the last dose.

We concluded that accumulation in PMNNs is high and that the $t_{1/2}$ in PMNNs and in sputum is long enough (289 h) to explore a dosing interval of 1 week, assuming a relationship between concentration in PMNNs or sputum and the clinical efficacy of AZM [7].

The key mode of action of AZM in CF patients remains unclear. Both a sub-MIC anti *Pseudomonas* effect due to interference with *Pseudomonas* biofilm formation or a non-

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antimicrobial anti-inflammatory effect have been hypothesized to lead to a reduction in decline of lung function [8–11]. Several investigators have published results of in vitro experiments towards the mode of action and the corresponding concentrations of AZM. Tateda et al. found inhibition of quorum sensing factor production at concentrations of 2 mg/l in the *P. aeruginosa* strain PAO1 and a reduction of the viability of *P. aeruginosa* after exposure of 48 h of AZM in a concentration of 0.5 mg/l [8,9]. Takeoka et al. found a MIC of 100 mg/l towards both mucoid and non-mucoid strains of *P. aeruginosa*. Moreover this study also suggested an increase of *P. aeruginosa* phagocytosis by PMNNs after exposure to sub MIC concentrations of AZM [10].

The sputum levels we found in our previous study exceed the levels at which quorum sensing signalling is reported to be influenced but do not reach MIC levels towards *P. aeruginosa* [7]. Based on our previous findings at a dose of 500 mg/day, we postulate that sputum levels will remain at a level high enough to retain the proposed anti *Pseudomonas* and/or anti-inflammatory activity during a dosing schedule with an extended dosing interval.

The weekly dose selected in the current study was primarily based on equivalence to the lowest cumulative weekly dose administered in a randomized controlled trial and secondly a weekly dose of 1000 mg is generally well tolerated in other patient groups (i.e. prophylactic treatment in HIV-infected patients and the treatment of *Chlamidia trachomatis* infection) [12,13].

The primary aim of this study was to describe pharmacokinetic data and exposure in blood, PMNNs and sputum of a once weekly dosing schedule in CF patients already on chronic once daily AZM treatment and to compare data of both dosing schedules. A secondary target is to describe the tolerability of the once weekly dosing schedule. The clinical efficacy of such a change in dosing regimen was not a part of this study.

2. Patients and methods

2.1. Patients

8 adult patients with CF were recruited from the Adult Cystic Fibrosis Centre at the Haga Teaching Hospital. Patients receiving AZM 500 mg once daily for at least 35 days with chronic *P. aeruginosa* infection (confirmed with at least 2 positive cultures in the last 6 months) were eligible to the study. The study protocol was approved by the regional medical ethical review board and patients gave written informed consent prior to study participation.

2.2. Drug administration and sampling

AZM was administered as 500 mg tablets (Zithromax® tablets 500 mg, Pfizer, Capelle a/d IJssel, The Netherlands) once daily for at least 35 days prior to recruitment in the once weekly study. Upon enrolment, blood samples were collected immediately before (C_0) and 3 h after (C_3) the final dose of 500 mg AZM once daily. Sputum was collected during 24 h. Hereafter the dosing regimen was changed to 1000 mg once weekly and continued throughout the 3-month study period. Blood and sputum samples were collected after 1, 2 and 3 months. Spontaneously produced sputum was collected during a 24-h period preceding the blood collection. After the 3-month study, patients returned to 500 mg AZM daily.

At each time point blood samples were collected immediately before the weekly dose of AZM and 3 h after dosing. AZM was given in the form of two 500 mg tablets which were swallowed with a glass of water.

Venous lithium-heparinized blood (119 IU li-heparin/7-ml tube, Vacutainer™ Becton-Dickinson, Alphen a/d Rijn, The Netherlands) samples were collected at each time point.

Adverse effects were documented at each visit as a measure of drug tolerability.

2.3. Isolation procedure of polymorphonuclear neutrophils and bioanalysis of AZM

PMNNs were isolated from lithium-heparinized venous blood samples. Before the separation a differential blood cell count was made. To 6.0 ml of blood 6.0 ml of phosphate-buffered saline (PBS; pH 7.4, Mallinckrodt-Baker, Deventer, The Netherlands) was added. The diluted blood–PBS mixture was transferred into a separation tube with a 6.0-ml layer of Ficoll-Paque Plus® density separation medium (Amersham Biosciences, Uppsala, Sweden) and centrifuged for 15 min (1250 G) at 21 °C. The supernatant and Ficoll-Paque Plus® layer were removed and the cell pellet with neutrophils and erythrocytes was incubated during 15 min with 45 ml of NaCl 0.2% at 2–6 °C in order to lyse the erythrocytes. PMNNs were isolated by centrifugation (5 min 465 G) and resuspended in 6.0 ml PBS. A differential cell count was performed to determine the number of isolated neutrophils. After centrifugation (5 min 465 G) the supernatant was removed and the cell pellet was kept at −30 °C until assay of AZM.

Detailed specifications and validation of the methodology of cell isolation and AZM quantification in blood, plasma and PMNNs are described elsewhere [14].

In brief, a high-performance liquid chromatographic method with pre-column derivatization and fluorescence detection was used for the quantification of AZM in blood, plasma, isolated PMNNs and sputum. Clarithromycin (CLM, Abbott, Queenborough, UK) was used as an internal standard and AZM dihydrate salt (Pfizer Inc., New York, USA) was used as reference. Pre-column derivatization was performed using 9-fluorenylmethyloxycarbonyl-chloride (Sigma-Aldrich, Zwijndrecht, The Netherlands). Analytical separation was carried out using a C18 column as stationary phase and a mixture of 760 ml acetonitrile (Merck, Darmstadt, Germany) and 240 ml 0.02 M phosphate buffer (0.65 g potassium dihydrogen phosphate (Merck, Darmstadt,