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Pharmaceutical development and optimization of azithromycin suppository for paediatric use

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ABSTRACT

Pharmaceutical development and manufacturing process optimization work was undertaken in order to propose a potential paediatric rectal formulation of azithromycin as an alternative to existing oral or injectable formulations. The target product profile was to be easy-to-use, cheap and stable in tropical conditions, with bioavailability comparable to oral forms, rapidly achieving and maintaining bactericidal concentrations. PEG solid solution suppositories were characterized *in vitro* using visual, HPLC, DSC, FTIR and XRD analyses. *In vitro* drug release and *in vivo* bioavailability were assessed; a study in rabbits compared the bioavailability of the optimized solid solution suppository to rectal solution and intra-venous product (as reference) and to the previous, non-optimized formulation (suspended azithromycin suppository). The bioavailability of azithromycin administered as solid solution suppositories relative to intra-venous was 43%, which compared well to the target of 38% (oral product in humans). The results of 3-month preliminary stability and feasibility studies were consistent with industrial production scale-up. This product has potential both as a classical antibiotic and as a product for use in severely ill children in rural areas. Industrial partners for further development are being sought.

1. Introduction

Bacterial infections still take a heavy morbidity and mortality toll on the lives of children, particularly those under 5 years of age (WHO, 2012). Against this scenario, there is a lack of paediatric formulations of antibiotics that are adapted to the needs of the developing world, where these infections are mostly prevalent. Particularly needed are formulations that can be administered by unqualified personnel to children who cannot take oral medications ("non-per-os") because their conditions are deteriorating.

The desired target product profile (TPP) was: (i) an antibiotic with a spectrum of action covering the main agents causing paediatric infections; (ii) use in both uncomplicated and complicated cases (where oral administration is not possible (patient "non-per-os")); (iii) safe and easy to use by untrained personnel; amenable

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to near-home use; (iv) cheap; (v) stable in tropical conditions of temperature and humidity.

Work conducted in our laboratory (Kauss et al., 2012) had investigated options for a paediatric rectal formulation of azithromycin (AZ). The rectal route is acceptable in the targeted countries (Simba et al., 2009; Thera et al., 2007), and can be used in both uncomplicated and complicated ("non-per-os" cases); there is evidence, for instance, that rectal artesunate can save lives in case of malaria (Gomes et al., 2009). AZ (a macrolide) was considered as a drug candidate for this indication because of its broad antibacterial spectrum of activity and pharmacokinetic properties (distribution and concentration in infected organs, prolonged half-life offering the convenience of once daily administration) (Jacobs et al., 2005; Langtry and Balfour, 1998; Van Bambeke and Tulkens, 2001). Among existing chemical forms of AZ, the AZ dihydrate was chosen because of its stability (Gandhi et al., 2002).

There is no rectal formulation of a macrolide on the market, and little information exists on their rectal availability. The reported rectal bioavailability would be acceptable for erythromycin (28–54% bioavailability, varying with age (Stratchunsky et al., 1991)) but very low for azithromycin (3.2% bioavailability (Bergogne-Bérézin and Bryskier, 1999)).

In order to improve AZ bioavailability (Kauss et al., 2012) we tried: (i) enhancing viscosity and muco-adhesiveness to prolong the residence time with rectal gels, (ii) dry form formulated as rectal capsule, and (iii) enhancing solubility with solid dispersion suppositories. Fatty-base suppositories were excluded because their low melting point makes them incompatible with tropical conditions. The most promising prototype was the suspended AZ PEG suppositories based on solid dispersion approach, which attained 28% bioavailability (Kauss et al., 2012). In a solid dispersion, active ingredients are dispersed in an inert carrier or matrix of solid state, prepared by melting, solvent or melting-solvent method (Chiou and Riegelman, 1971). Solid dispersions include eutectic mixtures, solid solutions, glass solutions and suspensions, amorphous precipitations, compound or complex formation, combinations and miscellaneous mechanisms (Chiou and Riegelman, 1971).

The aim of the current study was to optimize the AZ suppository formulation in order to enhance its rectal bioavailability and render it amenable to further development.

2. Materials and methods

2.1. Materials

Azithromycin dihydrate (AZ) was generously donated by Pfizer (France). Zithromax® (Pfizer, USA) was used as IV AZ formulation.

Pharmaceutical excipients, namely polyethylene glycol (PEG) 1500 and 4000 purchased from Fagron (Spain) and Miglyol 812N (Inresa, France) were of pharmaceutical grade. All other chemicals and solvents were of analytical reagent grade. Water was purified and deionized by the Millipore® Simplicity system (USA).

2.2. Suppository preparation

All the tested formulations except IV (Zithromax®, Pfizer) were developed in our laboratory.

All suppositories were prepared using the moulding method.

To obtain suspended AZ suppositories, PEGs were melted at 65 °C in a water bath and then AZ was dispersed under mechanical stirring at 150 rpm.

For co-melted AZ suppositories, PEGs were pre-melted at high temperature above the melting points of all constituents (130 °C) in an oven (Jouan Paris, France) and then AZ was added. The preparation was left in the oven until AZ was melted and stirred to obtain a homogeneous blend.

Finally, in the case of solid solution AZ suppositories, PEGs were melted at 90 °C in a water bath and AZ was added. The mixture was stirred until obtaining a homogenous limpid blend.

For all suppositories, the blend was cooled to a temperature of 55–60 °C before being poured in 2 g suppository moulds. They were allowed to harden at room temperature in a dessicator for at least 24 h and stored in individual alu/alu blister or kept in their plastic moulds in a fridge before further analysis or use.

While screening different options for the feasibility of solid solution suppositories, the AZ content was varied and changes compensated with the PEG mixture content (qsf 100%, while keeping the ratio PEG 1500/PEG 4000 constant). For manufacturing optimization, the manufacturing process was varied while keeping the formulation unchanged. For scale-up studies, batch quantities were increased while the process and the formulation remained the same.

2.3. In vitro pharmacotechnical controls of AZ PEG suppositories

Suppositories were visually evaluated at each withdrawal and in each storage condition; the colour, the limpidity and consistency were taken into account. The melted aspect of suppositories was also observed on a watch glass. For this purpose, crushed suppository aliquot of approximately 50 mg was melted at 70 °C in an oven (Jouan-Paris, France) on the watch glass.

Dissolution behaviour of AZ suppositories ($n = 6$ for each formulation and each storage condition) was compared using the Pharmacopoean II apparatus (SOTAX AT 7, Switzerland). Suppositories were introduced in bowls containing 250 ml of phosphate buffer (50 mM) pH 7.0 maintained at 37.0 °C ± 0.2 and 75 rpm. Samples (1 ml aliquot replaced by an equal volume of fresh dissolution medium) were withdrawn at time 0, 15, 30, 45, 60, 90 and 120 min using a 10 μm porous prefilter. Samples were diluted suitably using phosphate buffer before HPLC analysis.

AZ drug content was determined using HPLC method described beneath. A 65 mg of crushed suppository was dissolved in 20 ml with HPLC mobile phase. After 10 min of magnetical stirring, the filtered preparation was analyzed without further dilution.

2.4. HPLC analysis of azithromycin

AZ content and dissolution profile were determined by an HPLC system composed of 515 HPLC Pump Waters, Waters 2487 Dual λ Absorbance Detector and Waters 717 plus Autosampler (Waters, France). Data were managed using Millenium^{32} Chromatography manager (Waters, France). HPLC method was based on Gaudin et al. (2011) method. AZ was eluted using Luna C8 EC 5 μm, 150 mm × 4.6 mm column (Phenomenex, France) thermostated with Crococil oven (CIL, Saint Foy la Grande, France) at 45 °C. The mobile phase at apparent pH of 9 was composed by methanol/phosphate buffer 15 mM (80/20, v/v) at flow rate of 1.2 ml min^{-1}. The injected volume was 10 μl and AZ was detected at 215 nm.

2.5. Differential scanning calorimetry (DSC) analysis

DSC analysis was performed using a differential scanning calorimeter (Mettler Toledo TA controller and DSC30, Switzerland) with STAR^e software. DSC method consisted in a heating rate of 5 °C min^{-1} in the range of 30–180 °C for thermograms, or 2 °C min^{-1} in the range of 30–150 °C for melting point determination ($n = 3$ for each formulation). Precisely weighted samples of 6–8 mg were in aluminium pans, sealed and perforated with a pin. An empty sealed perforated aluminium pan was used as reference. For comparison,