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Comparison of 2 strategies to enhance pyridoclax solubility: Nanoemulsion delivery system *versus* salt synthesis

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ABSTRACT

Pyridoclax is an original oligopyridine lead, very promising in treatment of chemoresistant cancers. However, from solubility measurement and permeability evaluation, it appeared that this compound can be considered as a BCS II drug, with a poor water solubility. To overcome this unfavorable property, two strategies were proposed and compared: pyridoclax di-hydrochloride salt synthesis and formulation of pyridoclax-loaded nanoemulsions (PNEs) efficiently performed by transposing the spontaneous emulsification process previously developed by our team. Whereas the salt improved the thermodynamic solubility of the drug by a factor 4, the apparent solubility of the encapsulated pyridoclax was 1000-fold higher. Their stability was assessed upon dilution in various complex biomimetic media relevant for oral administration (SGF, FaSSIF-V2, FeSSIF-V2) or for the intravenous route (PBS). The solubility of the salt was affected by the nature of the medium, indicating that it could precipitate after administration, negatively impacting its bioavailability and its efficiency *in vivo*. On the contrary, in all media, PNEs remained stable in terms of granulometric properties (determined by DLS), ζ-potential and encapsulation efficiency (measured by HPLC). Thus, such nanomedicines appear as a valuable option to perform preclinical studies on the promising pyridoclax.

1. Introduction

Pyridoclax is an original oligopyridine recently designed and synthesized which could be particularly interesting in treatment of chemoresistant cancers. Indeed, it was established that without being cytotoxic when administered as a single agent, pyridoclax directly binds to Mcl-1, and hence sensitizes ovarian carcinoma cells to Bcl-x_L-targeting strategies. Thus, it induces apoptosis in ovarian, and also in lung, and mesothelioma cancer cells when it is administrated in combination with Bcl-x_L-targeting siRNA or Bcl-x_L targeting molecules such as ABT-737 or its orally available derivative ABT-263 (navitoclax) (Gloaguen et al., 2015).

However, the drugability parameters of this lead could hamper its preclinical development. Indeed, *in silico* assessment suggests a lipophilic structure (estimated logP = 5.64 from ACD/Labs on Chemspider website, http://www.chemspider.com). Numerous aromatic rings are present, and in addition, there is no ionizable group at pH = 7.4 and no H-bond donor. Considering the infringement of Lipinsky's 'Rule of Five' (Lipinski et al., 1997), pyridoclax is likely to have oral absorption/permeability issues. From that, at this drug discovery stage, it appears essential to precisely determine the thermodynamic solubility of pyridoclax and its permeability, and to propose, if necessary, valuable solubility/permeability enhancement strategies before envisaging preclinical studies in animals.

From the "Biopharmaceutics Classification System", poorly water soluble compounds can be categorized as BCS class II or IV drugs (Amidon et al., 1995). To enhance their solubility in gastrointestinal fluids, and hence, to improve their bioavailability, various solubilization approaches can be proposed. They include chemical strategies based on salt formation and prodrug synthesis (Ma et al., 2016), or advanced formulation strategies such as use of surfactants, dendrimers (Goldberg et al., 2011), drug-cyclodextrin inclusion complexes (Kurkov and Loftsson, 2013), mesoporous systems (Xu et al., 2013), solid dispersions (Yang et al., 2015), polymer nanoparticles (Hens et al., 2015), and Lipid-Based Drug Delivery Systems (LBDDS) (Mu et al., 2013).

LBDDS are recognized as one of the most promising methods to improve absorption of lipophilic drugs (Cerpnjak et al., 2013). Indeed, they can increase their solubilization while preventing their precipitation upon dilution in the gastrointestinal (GI) tract, enhance their membrane permeability by inhibiting efflux transporters, reducing CYP enzymes activity, increasing chylomicron production and lymphatic transport (O'Driscoll and Griffin, 2008). LBDDS include lipid solutions, suspensions or emulsions, self-emulsifying drug delivery systems (SEDDS) or self-microemulsifying drug delivery systems (SMEDDS), solid lipid nanoparticles or lipid nanocapsules, and liposomes. They have attracted

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much attention in the last 20 years thanks to their biocompatibility, their ease of preparation, their versatility, and above all, their capacity to improve the bioavailability of poorly water soluble drugs. For example, the bioavailability of paclitaxel was 3-fold improved when it was loaded in lipid nanocapsules (Groo et al., 2015).

Recently, a versatile lipid nanoemulsion (NE) formulation was developed (Gué et al., 2016). NE are highly biocompatible, and isotropic dispersed systems consisting of nanoscale oil droplets (typically, 20–300 nm). The developed formulation was shown to efficiently encapsulate drugs with different physico-chemical profiles. Moreover, its intrinsic properties in terms of droplets size, pH, osmolarity, sterility, have been optimized to render the NE administrable by different routes, implying the oral and the intravenous (i.v.) ones. In drug discovery, such NEs provide a valuable option as formulation strategy to improve properties of promising leads.

As Li and Zhao underline, the importance of such early formulations should never been underestimated since they contribute to the effective selection of future possible drug candidates (Li and Zhao, 2007). Therefore, soon after lead identification, it is recommended to start a pharmaceutical evaluation (Balbach and Korn, 2004). In particular, soon after early formulation development and before preclinical studies, it is useful to assess its stability, in particular upon dilution (Li and Zhao, 2007). Indeed, once the formulation is administered into the body after oral or i.v. bolus injection, supersaturation generated from its dilution with aqueous biological media fluids (blood, gastric fluid, *etc.*) may be responsible for precipitation of the drug. The precipitated drug or particulates can imply pain, thrombophlebitis, unreproducible and/or decreased bioavailability. In order to obtain relevant information, dilution factor and media must be chosen to be the most closed to the physiologic conditions.

Considering these points, in the present article, we propose to experimentally define drugability parameters relevant for bioavailability of the promising pyridoclax, *i.e.* solubility and permeability. Two strategies will be proposed to overcome its unfavorable properties: the chemical synthesis of a pyridoclax di-hydrochloride salt and the development of pyridoclax-loaded nanoemulsions by transposing the process previously developed by our team (Gué et al., 2016). These approaches will be compared in terms of improved apparent solubility. Moreover, their stability will be assessed upon dilution in various biomimetic media relevant for oral administration or for the intravenous route.

2. Materials and methods

2.1. Materials

$Na_2HPO_4 \cdot 2H_2O$, $Na_2HPO_4 \cdot H_2O$, $NaH_2PO_4 \cdot 12H_2O$, K_3PO_4, K_2HPO_4, and KH_2PO_4 were obtained from Sigma-Aldrich (Steinheim, Germany). Labrafac® CC (caprylic/capric acid triglycerides) and Labrasol® (caprylocaproyl macrogol-8 glycerides) were kindly provided by Gattefossé S.A. (Saint-Priest, France). Kolliphor® HS15 (70% PEG 660 hydroxystearate and 30% free PEG 660) was a gift from BASF AG (Ludwigshafen, Germany). Methanol, acetonitrile, water of HPLC grade, formic acid, sodium hydroxide, hydrochloric acid were provided by Prolabo VWR International (Fontenay-sous-Bois, France). Sodium chloride was obtained from Carlo Erba (Val de Reuil, France). Pepsin from porcine gastric mucosa, sodium taurocholate hydrate, 1-oleoyl-*rac*-glycerol, sodium oleate, maleic acid, and pancreatin (>3 × USP specification) were purchased from Sigma-Aldrich (Steinheim, Germany). Lipoid E PC (Phosphatidyl choline from egg lecithin) was a gift from Lipoid GmbH (Ludwigshafen, Germany). Calcium chloride was purchased by Prolabo VWR International (Fontenay-sous-Bois, France). Pyridoclax was synthesized according to the process previously described (Gloaguen et al., 2015; Voisin-Chiret et al., 2009).

2.2. Methods

2.2.1. Pyridoclax salt

2.2.1.1. Pyridoclax salt synthesis. A freshly prepared solution of diethyl ether bubbled into hydrochloric acid 37% (12 mL, 50:50; v/v) was dropwise added to a reaction vessel containing pyridoclax (400 mg, 0.87 mmol) in dichloromethane (20 mL) obtaining precipitation of the salt as a yellow solid. The final product was filtered on Goosh, washed with diethyl ether and dried in oven at 50 °C for 24 h, affording pure compound in quantitative yield.

2.2.1.2. Pyridoclax salt physico-chemical characterization. Melting points (Mp) were determined on a Köfler apparatus. Nuclear Magnetic Resonance (NMR) spectra were recorded at 400 MHz (Bruker Avance III 400 MHz) for ^1H NMR, at 100 MHz for ^{13}C NMR in DMSO-d_6 with chemical shift (δ) given in parts per million (ppm) relative to TMS as internal standard and recorded at 295 K. The following abbreviations are used to describe peak splitting patterns when appropriate: s = singlet, d = doublet, t = triplet, m = multiplet, dd = doublet of doublet, dt = doublet of triplet, ddd = doublet of doublet of doublet. Coupling constants *J* are reported in hertz units (Hz). Infrared spectra (IR) were obtained on a PERKIN-ELMER FT-IR spectrometer and are reported in terms of frequency of absorption (cm^{-1}) using KBr discs. Liquid Chromatograph-Mass Spectrum ElectroSpray Ionization (LC-MS (ESI)) analyses were realized with Waters Alliance 2695 as separating module using the following gradients: A (95%)/B (5%) to A (5%)/B (95%) in 4.00 min. This ratio was hold during 1.50 min before return to initial conditions in 0.50 min. Initial conditions were then maintained for 2.00 min (A = H_2O, B = acetonitrile; each containing formic acid: 0.1%; column XBridge C18 2.5 μm/4.6 × 50 mm; flow rate 0.8 mL/min). MS were obtained on a SQ detector by positive ESI. Mass spectrum data are reported as *m/z*.

2.2.2. Thermodynamic solubility determination

Thermodynamic solubility at pH 7.4 of pyridoclax and salt was determined according to the classical shake-flask method (Organization for Economic Cooperation and Development guideline n° 105) and a miniaturized procedure recently described (Bard et al., 2008). 10 mM phosphate buffered saline pH = 7.4, with an ionic strength of 154 mM, was prepared from Na_2HPO_4, NaH_2PO_4 and KCl. 10 μL of 25 mM of studied compound in DMSO was added to 1.5 mL microtube containing 990 μL of buffer solution ($n = 3$). Tubes were briefly sonicated and shacked by inversion at room temperature. An immediate precipitation was observed in all tubes. After 24 h, microtubes were centrifuged at 12.225*g* for 5 min; 100 μL supernatant was mixed with 100 μL acetonitrile/DMSO (99:1; v/v) in a Greiner UV microplate. Determination of solubility was made with a Synergy 2 (BioTek instrument, Colmar, France) in spectrophotometric mode ($\lambda_{max} = 286$ nm) from a calibration curve obtained from four standard solutions of pyridoclax and salt solubilized at 0, 12.5, 25, and 50 μM in a 50:50 (v/v) mixture of water or buffer with acetonitrile/DMSO (99:1; v/v). Calibration curves were linear with $R^2 > 0.999$. Standard solutions were prepared extemporaneously from stock solutions of 2 compounds solubilized in DMSO at 0, 2.5, 5, and 10 mM; 5 μL each stock solution was diluted with 995 μL buffer and then mixed with 1 mL acetonitrile to keep unchanged the final proportions of each solvent in standard solutions and samples.

2.2.3. Parallel artificial membrane permeability assay (PAMPA)

The PAMPA-GIT experiments were conducted using the Pampa Explorer Kit (Pion Inc., Billerica, USA) according to manufacturer's protocol. Each stock compound solution (10 mM in DMSO) was diluted in Prisma HT buffer pH = 7.4 (pION) to 50 μM. 200 μL of these solutions ($n = 4$) were added to the donor plate (P/N 110243). 5 μL of the GIT-0 Lipid (P/N 110669) was used to coat the membrane filter of the