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The effect of macrophage and angiogenesis inhibition on the drug release and absorption from an intramuscular sustained-release paliperidone palmitate suspension

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ABSTRACT

The intramuscular (IM) administration of long-acting injectable (LAI) aqueous nano-/microsuspensions elicits a chronic granulomatous injection site reaction, which recently has been hypothesized to drive the (pro)drug dissolution and systemic absorption resulting in flip-flop pharmacokinetics. The goal of this mechanistic study was to investigate the effects of the local macrophage infiltration and angiogenesis on the systemic drug exposure following a single IM administration of a paliperidone palmitate (PP) LAI nano-/microsuspension in the rat. Liposomal clodronate (CLO) and sunitinib (SNT) were co-administered to inhibit the depot infiltration and nano-/microparticle phagocytosis by macrophages, and the neovascularization of the depot, respectively. Semi-quantitative histopathology of the IM administration sites at day 1, 3, 7, 14, 21 and 28 after dosing with PP-LAI illustrated that CLO significantly decreased the rate and extent of the granulomatous inflammatory reaction. The macrophage infiltration was slowed down, but only partially suppressed by CLO and this translated in paliperidone (PAL) plasma concentration–time profiles that resembled those observed upon injection of PP-LAI only, albeit with a lower PAL input rate and delayed maximum plasma concentration (C_{MAX}). Conversely, SNT treatment completely suppressed the granulomatous reaction, besides effectively inhibiting the neovascularization of the PP-LAI depot. This resulted in an even slower systemic PAL input with delayed and lower maximum PAL C_{MAX}. The reduced PP-LAI lymph node retention after CLO and SNT treatment, as well as pharmacokinetic drug–drug interactions were rejected as possible sources of the observed pharmacokinetic differences. The biphasic PAL plasma concentration–time profiles could best be described by an open first-order disposition model with parallel fast (first-order) and slow (sequential zero-first-order) absorption. The correlation of the pharmacokinetic data with the histopathological findings indicated that the macrophage infiltration, with subsequent phagocytosis of an important fraction of the PP-LAI dose, actively contributed to the observed PAL plasma exposures by promoting the prodrug dissolution and conversion to the active. An initial fast PP dissolution of individual nano-/microcrystals present in the interstitium was followed by a second, slower, but dominating input process that was driven by the PAL formation rate in the infiltrated portions of the LAI depot. The present work provides new fundamental insights into the influence of the local tissue response to IM LAI (pro)drug suspensions on the systemic drug exposure. This knowledge might support the future development of predictive *in vitro* and *in silico* models, which could help guide the LAI formulation design.

1. Introduction

The development of sustained-release drug formulations that are capable of providing stable therapeutic plasma concentrations for several weeks or months has been the subject of extensive investigation over the last decade [1]. Long-acting injectable (LAI) drug nano-/microsuspensions have relatively recently risen as a viable drug delivery strategy and are nowadays recognized as an attractive formulation option for poorly water-soluble drugs intended for chronic therapy. LAI nano-/microsuspensions consist of nano-sized or micron-sized (*i.e.* typically <10 μm) pure drug crystals dispersed in an aqueous vehicle containing hydrophilic stabilizing excipients [2]. Most of the benefits of nano- or microsuspensions originate from their high mass-specific surface area, the high drug contents (*cf.* absence of drug carrier) and

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the organic solvent-free and industrially feasible manufacturing process, which all have been extensively reviewed elsewhere [3–5]. The success of this formulation approach has been reflected by the marketing authorization of 3 LAI nano-/microsuspensions within the last 6 years, with several others currently undergoing clinical evaluation [6–9].

The *in vivo* drug absorption from sustained-release suspensions of poorly soluble compounds is conventionally thought to be dissolution rate-limited [10]. Hence, the drug release can generally be prolonged by adjusting the particle size and the compound's aqueous solubility (*e.g.* by lowering the solubility by salt or prodrug formation) [1,11]. Lipophilic prodrug esters (*e.g.* palmitoyl esters) dissolve very slowly due to their extremely low aqueous solubility, but are rapidly cleaved by esterases present at the injection site prior to systemic absorption [12,13].

Hirano et al. and Zuidema et al. have provided in the 1980s solid experimental and mathematical evidence of the dissolution rate-controlled drug absorption following intramuscular (IM) injection of aqueous suspensions of poorly water-soluble drugs [14–16]. The drug absorption rate was said to be predominantly controlled by the physical behavior of the formulation itself. The degree of particle agglomeration and the spreading, both strongly influenced by the particle size, dictate the effective dissolution surface area and, therefore, the drug absorption rate [10]. Although these models presented a significant step forward, they fall short of providing a comprehensive model that would allow a rational development of LAI aqueous suspensions. Despite the fact that the drug dissolution and absorption processes are undeniably determined by the formulation properties, they can also be affected by interactions of the surrounding tissues with the foreign drug delivery systems [17–19].

In an effort to better understand the *in vivo* mechanisms that contribute to the observed (often complex) pharmacokinetics (PK) of LAI nano-/microsuspensions, our group recently investigated the local disposition and PK of an IM administered paliperidone palmitate (PP) nano-/microsuspension in the rat [20]. The elicited injection site reaction consisted of an acute inflammation that was followed by a chronic inflammatory reaction. Large amounts of crystalline PP-LAI particles were found within the infiltrating macrophages. This was accompanied by biphasic plasma PK of paliperidone (PAL, *i.e.* active moiety of PP); a phenomenon that has also been reported in humans, as well as for other LAI nano- or microsuspensions [7,12,21]. The time-course of the local response to IM PP-LAI, which was mainly composed of a granulomatous inflammatory reaction with extensive infiltration and sequestration of the formulation depot by macrophages and local neovascularization, was (semi-)quantitatively characterized in a subsequent study [22]. Surprisingly, the temporal evolution of the volume of the PP-LAI depot that had been infiltrated by macrophages and that was relocated intracellularly, qualitatively resembled that of the PAL plasma concentration–time profiles observed upon IM administration of PP-LAI. It was thus hypothesized that the intracellular accumulation of major fractions of the PP-LAI dose in macrophages could result in the formation of a secondary PP-LAI depot, from which the prodrug (PP) dissolution and drug (PAL) formation would occur at different rates than under inflammation-free conditions. Another possible determinant of the PAL systemic input rate is the granuloma formation, which could be detrimental to the drug absorption [18]. Conversely, the neovascularization of the granuloma might provide additional systemic or lymphatic absorptive capacity [20,22]. Finally, the relocation of a portion of the administered dose to draining lymph nodes could possibly influence the plasma PK by presenting an alternative systemic drug absorption pathway. The formation of such secondary depots has been suggested in the case of a subcutaneously (SC) administered 200 nm rilpivirine LAI in dogs and was recently demonstrated for PP-LAI in the rat [7].

Despite the growing evidence of an inflammation-mediated modulation of the (pro)drug release and/or systemic absorption, satisfactory mechanistic explanations are still missing and the rate-limiting processes remain to be formally identified. The aim of the current study was to investigate the effect of the extensive macrophage infiltration and the local angiogenesis that occur following IM injection of a PP-LAI nano-/microsuspension, on the PAL plasma PK in rats. To this end, liposome-encapsulated clodronate (CLO, a non-nitrogenous bisphosphonate) was intermittently co-administered to inhibit the recruitment of macrophages towards the injection site and their subsequent infiltration and sequestration of the LAI formulation depot. Liposomal formulations of CLO have widely been applied to achieve selective macrophage depletion *in vivo* [23]. Sunitinib (SNT), a potent vascular endothelial growth factor receptor (VEGFR) antagonist and multiple receptor tyrosine kinase (RTK) inhibitor, was co-administered in another test group to inhibit the local neovascularization of the PP-LAI formulation depot [24]. Appropriate controls that received a single IM PP-LAI injection with or without co-administration of blank liposomes, were included in the study design. The effects of CLO and SNT on the injection site reaction were assessed by semi-quantitative histopathological evaluation of the IM administration sites on day 1, 3, 7, 14, 21 and 28 after dosing with PP-LAI according to a previously established methodology [20,22]. The lymph node retention of PP-LAI was evaluated by means of polarized light microscopy. The plasma pharmacokinetics of PP (prodrug) and PAL (active) were determined in all groups and non-compartmental PK analysis was performed. The disposition parameters were correlated with the histopathological findings. A population PK model describing the PAL disposition was developed to support the identification of covariates affecting the PK.

2. Materials and methods

2.1. Materials

Paliperidone palmitate racemate (PP; PubChem CID: 9852746), paliperidone racemate (PAL; PubChem CID: 115237), the internal standards JNJ-3905343 and JNJ-17340362, and Xeplion® (PP-LAI) were provided by Janssen Pharmaceutica NV (Beerse, Belgium). Sunitinib malate was supplied from LC Laboratories (Woburn, MA, USA). The clodronate disodium-containing liposomes and phosphate buffered saline-containing liposomes were ordered from clodronateliposomes.com (Haarlem, The Netherlands). Tween® 80 was obtained from AppliChem GmbH (Darmstadt, Germany). Citric acid monohydrate and sodium chloride were acquired from Chem-Lab NV (Zedelgem, Belgium). Anhydrous sodium acetate was ordered from MP Biomedicals Inc. (Solon, OH, USA) and low-viscosity carboxymethylcellulose sodium from Calbiochem–EMD Millipore Corp. (Billerica, MA, USA). Benzyl alcohol was purchased from Certa NV (Braine-l'Alleud, Belgium). VWR International BVBA (Leuven, Belgium) supplied glacial acetic acid. Type I (>18 MΩ) deionized water was obtained from an Elgastat Maxima system (Elga LabWater VWS Ltd., High Wycombe, UK). All chemicals and solvents were of analytical and HPLC grade, respectively.

2.2. Formulations

A PAL aqueous solution (PAL-IR) was obtained by dissolving 2.5 mg/ml in 10 mM sodium acetate–acetic acid (VWR International BVBA, Leuven, Belgium) buffer pH 4.0 at room temperature. The solution was filtered through a 0.2 μm Filtropur polyethersulfone membrane filter (Sarstedt AG & Co., Nümbrecht, Germany) directly into a sterile 10 ml injection vial and stored protected from light at 4 °C prior to use *in vivo* within 48 h.

The test article was the Xeplion® once-monthly IM injectable drug product. It consisted of a sterile long-acting injectable (LAI) aqueous suspension of PP nano-/microcrystals ($D_{V,50}$ = 1.08 μm) at a concentration of 156 mg/ml (PP-LAI) and contained no more than 2.33 I.U./mg endotoxin. The quantitative composition of the vehicle has been reported previously [20].The clodronate-containing (CLO) and phosphate buffered saline-containing (PBS) liposomes were used as obtained. The