International Journal of Pharmaceutics 529 (2017) 357–370

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

# Functionalized PLA-PEG nanoparticles targeting intestinal transporter PepT1 for oral delivery of acyclovir

Betty Gourdon[a,b,c], Caroline Chemin[a], Amélie Moreau[a], Thomas Arnauld[a], Philippe Baumy[a], Salvatore Cisternino[b,c], Jean-Manuel Péan[a], Xavier Declèves[b,c,*]

[a] Technologie Servier, 27 rue Eugène Vignat, Orléans, France
[b] Inserm, U1144, Paris, F-75006, France
[c] Université Paris Descartes, UMR-S 1144, Faculté de Pharmacie de Paris, Paris, F-75006, France

ARTICLE INFO

*Article history:*
Received 24 March 2017
Received in revised form 5 July 2017
Accepted 8 July 2017
Available online 10 July 2017

*Keywords:*
PepT1 transporter
Polymer nanoparticles
Nanoprecipitation
Dipeptide ligand
Intestinal absorption
Acyclovir

ABSTRACT

Targeting intestinal di- and tri-peptide transporter PepT1 with prodrugs is a successful strategy to improve oral drug bioavailability, as demonstrated with valacyclovir, a prodrug of acyclovir. The aim of this new drug delivery strategy is to over-concentrate a poorly absorbed drug on the intestinal membrane surface by targeting PepT1 with functionalized polymer nanoparticles. In the present study, poly(lactic acid)-poly(ethylene glycol)-ligand (PLA-PEG-ligand) nanoparticles were obtained by nanoprecipitation. A factorial experimental design allowed us to identify size-influent parameters and to obtain optimized ≈30 nm nanoparticles. Valine, Glycylsarcosine, Valine-Glycine, and Tyrosine-Valine were chemically linked to PLA-PEG. In Caco-2 cell monolayer model, competition between functionalized nanoparticles and [$^3$H]Glycylsarcosine, a strong substrate of PepT1, reduced [$^3$H]Glycylsarcosine transport from 22 to 46%. Acyclovir was encapsulated with a drug load of ≈10% in valine-functionalized nanoparticles, resulting in a 2.7-fold increase in permeability as compared to the free drug. An *in vivo* pharmacokinetic study in mice compared oral absorption of acyclovir after administration of 25 mg/kg of valacyclovir, free or encapsulated acyclovir in functionalized nanoparticles. Acyclovir encapsulation did not statistically modify AUC or $C_{max}$, but increased $t_{1/2}$ and MRT 1.3-fold as compared to free acyclovir. This new strategy is promising for poorly absorbed drugs by oral administration.



## 1. Introduction

With the development of peptides as efficient therapeutics, potential strategies multiplied and diversified for increasing oral bioavailability of hydrophilic and poorly absorbed drugs. The bioavailability of these molecules depends on their ability to cross the intestinal epithelium and reach the systemic blood circulation (Johnson, 1994; Kwan, 1997). Formulation strategies have been developed in the past decades in order to increase their intestinal uptake, such as absorption enhancers or mucoadhesive systems (Aungst, 2012; Williams and Barry, 2004; Longer et al., 1985; Shaikh et al., 2011). Finally, targeting specialized intestinal cells allowed a more efficient oral delivery of drugs (Russell-Jones, 2004; Varma et al., 2010). This method was widely explored with the use of prodrugs targeting either intestinal receptors or transporters (Majumdar and Mitra, 2006; Sun, 2010). Among them, PepT1, which is well known to promote the uptake of dipeptides, tripeptides and peptidomimetics due to an inward proton gradient, is the most widely studied. Thanks to its broad substrate specificity, it is still an attractive transporter to target by modifying a drug having a low oral bioavailability with a peptide-like ligand allowing its intestinal transport by PepT1 into the bloodstream. Midodrine, gabapentin enacarbil, zanamivir and oseltamivir are some of the molecules that have been chemically modified to target PepT1 (Terada and Inui, 2004; Vig et al., 2013; Tsuda et al., 2006; Gupta et al., 2011; Wang and Wang, 2009). The most relevant example is valacyclovir (ValACY), an acyclovir (ACY) prodrug linked to valine. It was shown that 90% of ValACY was transported by PepT1, resulting in a 3- to 5-fold increase in ACY human oral bioavailability (Beauchamp et al., 1992; Yang, 2012; Soul-Lawton, 1995). Another strategy for intestinal targeting is the use of functionalized nanocarriers: the targeting ligand is then linked to the carrier (micelle, liposome, polymer nanoparticle) and not to the drug. Functionalized nanocarriers targeting transferrin, vitamin B12 or folate receptors have been widely studied for the

* Corresponding author at: Inserm UMR–S1144 Universités Paris Descartes et Paris Diderot, 4 avenue de l'observatoire, 75006 Paris, France.
*E-mail address:* xavier.decleves@parisdescartes.fr (X. Declèves).

http://dx.doi.org/10.1016/j.ijpharm.2017.07.024
0378-5173/

oral delivery of drugs (Du et al., 2013; Gosselin and Lee, 2002; Roger et al., 2012; Stella et al., 2000). Nevertheless, the potential of nanoparticles to target intestinal transporters has not been investigated yet. Even if nanoparticles have a particle size under 200 nm allowing them to go through the intestinal mucus layer and reach the targeted plasma membrane transporter, they are obviously not expected to cross the intestinal barrier by transport mechanism (Ensign et al., 2012). By targeting an intestinal transporter, the aim was rather artificially increase the residence time of loaded nanoparticles, and consequently the drug, close to the intestinal membrane surface, thus increasing its chance to cross the intestinal membrane by its own mechanism of membrane permeability. This strategy was recently proven to be effective using an external magnetic field to maintain magnetic carriers close to the intestinal membrane (Seth et al., 2014). In the present first proof-of-concept study, the ability to target an intestinal transporter with functionalized nanoparticles in order to improve the bioavailability of a poorly absorbed hydrophilic drug was explored. As PepT1 was largely studied in order to enhance oral drug absorption with a prodrug strategy, it was thus chosen for the current one. But the main challenge concerned poorly absorbed hydrophilic molecules which could not be designed as prodrugs targeting PepT1. For the latter, a new strategy consisting in encapsulating them into functionalized nanoparticles targeting PepT1 transporters was designed.

Polymer nanoparticles made from PLA, PLGA or PLA-PEG, safe biodegradable polymers, were frequently used in order to encapsulate hydrophilic molecules (Peltonen et al., 2004; Rodrigues et al., 1995). Moreover, by using a PLA-PEG polymer expressing a terminal amine function, various ligands can be linked on its polar end, as Pridgen et al. did with the antibody fragment Fc (Pridgen et al., 2013). Nanoparticles were obtained by a nanoprecipitation method (Fessi et al., 1989). Briefly, this method is based on a water miscible solvent, dissolving both polymers and active ingredient, and when added to the aqueous phase involves self-assembly of polymers and formation of nanoparticles.

First of all, formulation and process parameters influencing the particle size of PLA-PEG-$NH_2$ nanoparticles were studied. PLA-PEG-$NH_2$ polymer was chemically functionalized with Valine, Tyrosine-Valine, Valine-Glycine and Glycylsarcosine ligands – chosen according to their enzymatic stability, their ability to target PepT1 and their capacity of being coupled to the PLA-PEG-$NH_2$ polymer (Anand et al., 2003). The ability of functionalized nanoparticles to target PepT1 was then investigated. For that purpose competition experiments were carried out *in vitro* with a Caco-2 cell model. Finally, drug-loaded nanoparticles were prepared with PLA-PEG having the most relevant ligand. ACY was selected as a tool compound because of its capacity to be encapsulated in polymer nanoparticles and in light of the promising results obtained by the prodrug strategy with ValACY targeting PepT1 (Gupta et al., 2013; Patel et al., 2014). Based on the functionalized nanoparticles concept, we hypothesized that acyclovir when prepared as acyclovir-loaded nanoparticles with a dipeptide-targeting ligand would have better oral performance than when administered alone. By doing so, the dipeptide ligand would direct the acyclovir-loaded nanoparticles to PepT1 at the luminal membrane, increase its residence time at the membrane surface, and then allow acyclovir to be released from the nanoparticles with a greater driving force (i.e., concentration difference) for absorption than what might be achieved when administered alone. This approach might be useful for hydrophilic drugs such as ACY or those that cannot be chemically modified as prodrugs targeting directly PepT1, a strategy which is already quite well demonstrated and the most efficient way to improve oral bioavailability of hydrophilic drugs.

Transport experiments were conducted *in vitro* and *in vivo* in mice to conclude on the input of PepT1 targeting with functionalized nanoparticles to improve the transport of poorly absorbed drugs.

## 2. Materials and methods

### 2.1. Materials

Poly(lactic acid)-poly(ethylene glycol) (PLA-PEG), Poly(lactic acid)-poly(ethylene glycol)-NH-Boc (PLA-PEG-NH-Boc) and PLA-PEG-Valine-Boc (PLA (Mw: 10 kDa)-PEG (Mw: 1 kDa)) polymers were synthetized by Specific Polymers. H-Valine-Tyrosine-OH (H-Val-Tyr-Boc) was purchased from Bachem. 1-*E*thyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) was procured from Iris Biotech GmbH. Dichloromethane (DCM), pentane, orthophosphoric acid, dimethylsulfoxide (DMSO) and trifluoroacetic acid (ATFA) were purchased from Merck. Acyclovir (ACY) and Valacyclovir (ValACY) were obtained from Sigma Aldrich Chemical Sourcing and Apichem Technology, respectively. PLGA Resomer® RG502 and RG502H (7–17 kDa) were graciously provided by EVONIK, Kolliphor® P188 and P407 poloxamers by BASF. Tween 80 was purchased from Seppic. Methanol (MeOH) was obtained from VWR. Acetonitrile (ACN) was procured from Biosolve. Tangential filtration tubes Vivaspin® were purchased from Sartorius Stedim. 24-well plates Transwells and T75 flasks were obtained from Corning. L-Glutamine and penicillin/streptomycin (P/S) were purchased from Invitrogen. Fetal bovine serum (FBS) was obtained from Lonza. Trypsine was procured from Life Technologie. [$^3$H] Glycylsarcosine and [$^{14}$C]Sucrose were purchased from Moravek Biochemicals and Perkin Elmer, respectively. Boc-Glycine-Valine-OH (Boc-Gly-Val-OH), Glycylsarcosine (GlySar), Di-*tert*-butyl dicarbonate ($BOC_2O$), *N,N*-diisopropylethylamine (DIEA), hydroxybenzotriazole (HOBt), polyvinyl alcohol (PVA) Mowiol® 4–88, formic acid, ammonium formate, non-essential amino acids (NEAA), amphotericin B, bovine serum albumin (BSA), Dulbecco modified eagle medium (DMEM), Hanks buffer saline solution (HBSS), Lucifer Yellow (LY), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) and 2-(*N*-morpholino)ethanesulfonic acid (MES) were obtained from Sigma-Aldrich.

### 2.2. Nanoparticle preparation and characterization

#### 2.2.1. Preparation of nanoparticles

PLA-PEG-$NH_2$ drug-free nanoparticles were prepared by nanoprecipitation. Polymers were dissolved in DMSO. The aqueous phase solution was prepared dissolving a surfactant in water. The organic phase was added to the aqueous phase under stirring. DMSO and water were removed by successive tangential filtrations during 1 h at 700 × g until a 100 μL residual volume of nanoparticle suspension was obtained. Nanoparticles were re-suspended in aqueous phase and stocked as a suspension.

Formulation and process parameters were studied using a factorial experimental design with PLA-PEG-$NH_2$ drug-free nanoparticles. The studied parameters are presented in Table 1 and the experimental plan is given in Supplementary material (Table S1). The studied formulation parameters were the amount and ratio of polymers in the organic phase, the ratio of organic *versus* aqueous phases, the amount of surfactant in the aqueous phase and the surfactant type. The process parameters included needle diameter, stirring duration, stirring rate, and addition time. Each parameter was studied at 3 or 4 levels. Design of multivariate experiments and calculations were performed with the software NEMRODW®. Data collected were the median particle size $X_{50}$ (i.e. 50% of the particles are smaller and 50% are larger) and the $X_{99}$ (particle dimension corresponding to 99% of the cumulative undersize