European Journal of Pharmaceutical Sciences 45 (2012) 429–435

Contents lists available at SciVerse ScienceDirect

European Journal of Pharmaceutical Sciences

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

Liposomes as carriers for colchicine-derived prodrugs: Vascular disrupting nanomedicines with tailorable drug release kinetics

Bart J. Crielaard [1], Steffen van der Wal [1], Huong Thu Le, Aloïs T.L. Bode, Twan Lammers, Wim E. Hennink, Raymond M. Schiffelers, Marcel H.A.M. Fens, Gert Storm *

Department of Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences (UIPS), Utrecht University, Utrecht, The Netherlands

ARTICLE INFO

Article history:
Available online 1 September 2011

Keywords:
Liposomes
Colchicine
Colchicinoids
Cancer
Prodrugs
Vascular disrupting agents

ABSTRACT

Newly formed tumor vasculature has proven to be an effective target for tumor therapy. A strategy to attack this angiogenic tumor vasculature is to initiate local blood vessel congestion and consequently induce massive tumor cell necrosis. Vascular disrupting agents (VDAs) typically bind to tubulin and consequently disrupt microtubule dynamics. Colchicine and its derivatives (colchicinoids) are very potent tubulin binding compounds but have a narrow therapeutic index, which may be improved by employing a liposomal targeting strategy. However, as a result of their physicochemical properties, colchicinoids are problematic to retain in liposomes, as they are released relatively rapidly upon encapsulation. To overcome this limitation, two hydrolyzable PEGylated derivatives of colchicine were developed for encapsulation into the aqueous core of long-circulating liposomes: a moderately rapid hydrolyzing PEGylated colchicinoid containing a glycolic acid linker (prodrug I), and a slower hydrolyzing PEGylated colchicinoid with a lactic acid linker (prodrug II). Hydrolysis studies at 37 °C and pH 7.4 showed that prodrug I possessed relatively rapid conversion characteristics ($t_{1/2}$ = 5.4 h) whereas prodrug II hydrolyzed much slower ($t_{1/2}$ = 217 h). Upon encapsulation into liposomes, colchicine was released rapidly, whereas both PEGylated colchicine derivatives were efficiently retained and appeared to be released only after cleavage of the PEG-linker. This study therefore demonstrates that, in contrast to colchicine, these novel PEGylated colchicine-derived prodrugs are retained within the aqueous interior after encapsulation into liposomes, and that the release of the active parent can be controlled by using different biodegradable linkers.

1. Introduction

Newly formed angiogenic endothelium has become an important target for the design of anticancer agents (Carmeliet and Jain, 2000). As tumor vasculature develops relatively fast during early tumor growth, blood vessels appear immature, disorganized and imperfect, which makes them a vulnerable target for cancer therapy (Baluk et al., 2005; Thorpe, 2004). Vascular disrupting agents (VDAs) initiate local disruption of tumor endothelium by interfering with the immature vasculature, causing site-specific blood vessel congestion. As a consequence, tumor cells are deprived from nutrients and oxygen, leading to rapid and massive tumor cell necrosis (Kanthou and Tozer, 2007; Lippert, 2007; Tozer et al., 2005). Tubulin binding agents (TBAs), such as colchicine and the structurally and pharmacologically similar colchicinoids, display vascular disrupting activity by binding irreversibly to tubulin at its colchicine domain, thereby inhibiting tubulin dynamics and microtubule formation (Bhattacharyya et al., 2008; Ravelli et al., 2004). Because endothelial cells in the tumor vasculature rely more on tubulin than actin to maintain their cell shape, the binding of TBAs to tubulin leads to rapid rounding of these cells, resulting in loss of vascular integrity and, ultimately, to hemostasis (Jordan and Wilson, 2004; Thorpe, 2004). TBAs are considered to be useful in cancer therapy not only because of their ability to disrupt existing angiogenic tumor vasculature, but also because of their capabilities to induce mitotic arrest of tumor cells and inhibition of angiogenesis (Jordan and Wilson, 2004; Lippert, 2007; Pasquier et al., 2006; Pasquier and Kavallaris, 2008).

Colchicine is currently used clinically in low doses for the treatment of acute gout (Keith and Gilliland, 2007; Petersel and Schlesinger, 2007), familial Mediterranean fever (FMF) (Cerquaglia et al., 2005), and dermatologic (Bibas et al., 2005) and autoinflammatory diseases (Terkeltaub, 2009). However, the utility of colchicine for cancer therapy is currently limited, as only doses close to the maximal tolerated dose (MTD) can induce reduction in tumor blood perfusion leading to a high risk for toxicity (Baguley et al., 1991; Nihei et al., 1999).

* Corresponding author. Address: Department of Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences (UIPS), Utrecht University, P.O. Box 80082, 3508 TB Utrecht, The Netherlands. Tel.: +31 30 2537388; fax: +31 30 2517839.
E-mail address: G.Storm@uu.nl (G. Storm).
[1] These authors contributed equally to this work.

0928-0987/$ - see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejps.2011.08.027

A potential strategy for improving the therapeutic index of colchicine, i.e. limiting the side effects and improving the efficacy, may be the employment of a colloidal drug delivery system, such as long-circulating liposomes (Fenske and Cullis, 2008; Lammers et al., 2008). Advantages associated with the use of long-circulating liposomes include their prolonged circulation kinetics, their passive targeting properties, as well as the possibility to encapsulate both hydrophobic and hydrophilic drugs (Torchilin, 2005). However, it remains a challenge to formulate long-circulating liposomes that allow the retention of drugs with moderate lipophilicity, such as colchicinoids, while enabling appropriate release kinetics once accumulated in the target site (Coimbra et al., 2011). Colchicinoids localize mainly in the lipid bilayer and leak out readily, which makes it troublesome to control their retention and release rate (Kulkarni et al., 1997; Mons et al., 2000). A potential way to overcome this challenge is to make use of prodrugs, which are (inactive) bioreversible derivatives of the active drug but often possess different physicochemical properties than the parent compound (Coimbra et al., 2011).

After enzymatic and/or chemical conversion of the prodrug, the parent molecule with its original pharmacological activity is recovered (Rautio et al., 2008). If the prodrug is designed to be very hydrophilic, it may be encapsulated solely in the aqueous core of liposomes, thereby limiting its diffusion over the lipid bilayer. Only upon conversion of the prodrug in the aqueous interior, the parent drug molecule, in this case the colchicinoid, may be released from the liposome by its ability to diffuse over the lipid bilayer. A useful method for developing hydrophilic prodrugs of small molecular weight drugs is the conjugation with poly(ethylene glycol) (PEG). PEG has high aqueous solubility, it is non-toxic, non-immunogenic and non-antigenic (Bell et al., 2007; Parveen and Sahoo, 2006). In addition, by utilizing a PEG-linker that is susceptible to hydrolysis, PEGylated prodrugs that can be cleaved in the liposomal interior are created. The molecular structure of the linker can influence the rate of hydrolysis, and therefore, by employing different linkers, the rate of conversion to the active drug can be controlled (Roberts et al., 2002).

In the current study, two PEGylated colchicinoid prodrugs were synthesized and encapsulated into long-circulating liposomes. The presented strategy allows retention of the colchicinoids – in their prodrug form – in the aqueous core of the liposomes, and at the same time enables release of the cleaved prodrug from the liposome, at a rate that can be tailored by adjustment of the hydrolysis kinetics (Fig. 1). The hydrolysis rate is controlled by using a hydrolysable PEG-linker based on a primary alcohol, which is more prone hydrolysis, or a linker based on a secondary alcohol, which is more resistant to hydrolysis. Since colchicinoids are able to pass the lipid bilayer, the release rate of colchicinoid from the liposome is directly related to the cleavage rate of the colchicinoid prodrug. Obviously, the release kinetics of colchicinoids from the liposomes, once they have accumulated in the tumor, is a critical determinant for therapeutic activity. Therefore, a tailorable drug delivery system for colchicine derivates may prove of great value for improving the therapeutic index of colchicinoids in the therapy of solid tumors.

2. Material and methods

2.1. Materials

1,2-Distearoyl-sn-glycero-3-phosphoethanolamine-*N*-[methoxy (polyethylene glycol)-2000] (DSPE-PEG_{2000}) and dipalmitoylphosphatidylcholine (DPPC) were provided by Lipoid (Germany). All other chemicals were ordered from Sigma–Aldrich (Germany) and used without further purification. Phosphate Buffered Saline (PBS) with pH 7.4 was purchased from B. Braun (The Netherlands). Solvents were obtained from Biosolve (The Netherlands) and used without distillation. Acetonitrile and dichloromethane were stored on molecular sieves prior to use (3 and 4 Å, respectively). All reactions were performed without direct lighting and with flasks covered in aluminum foil to prevent degradation of colchicine. Flash chromatography was performed using silica gel of 0.035–0.070 mm 60 Å mesh (Acros Organics, Belgium). TLC analysis was performed using plastic backed silica 60 F_{254} plates (Merck, Germany) and NMR spectra were recorded on a Varian Gemini 300 MHz spectrometer. ESI mass analysis was performed on a Shimadzu LCMS-QP8000 single quadrupole spectrometer in positive ionization mode.

2.2. Synthesis of N-Boc-colchicine (2)

Colchicine (**1**, 4.9 g, 12.3 mmol) and DMAP (1.5 g, 12.5 mmol) were dissolved in acetonitrile (50 mL). Subsequently Boc_2O (17.4 g, 79.7 mmol), dissolved in acetonitrile (50 mL), and dry Et_3N (3 mL, 21.5 mmol) were added. The reaction mixture was refluxed for 1.5 h after which additional Boc_2O was added (1.6 g,

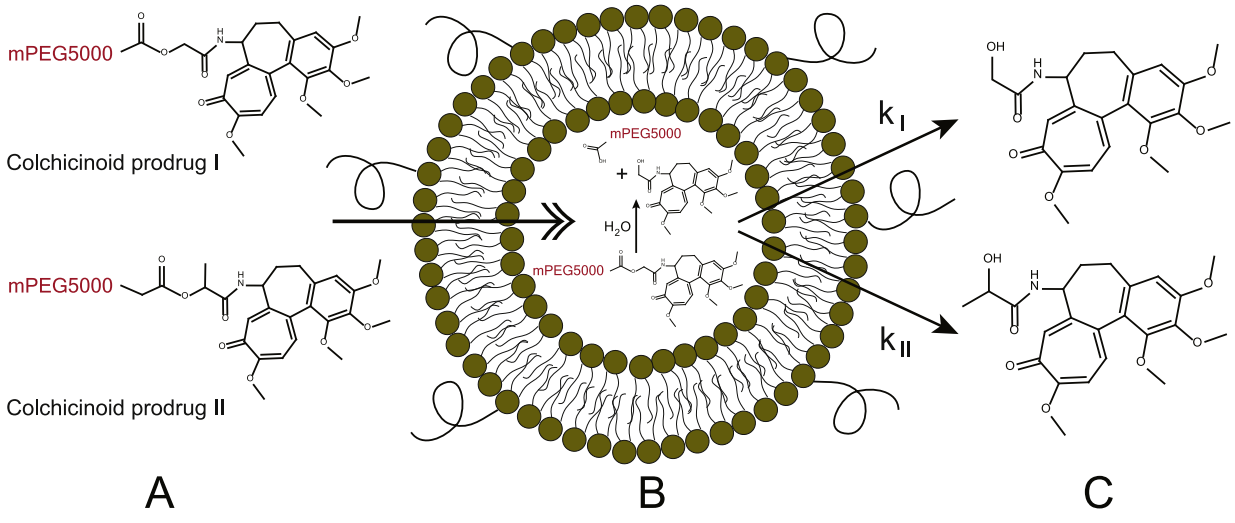

Fig. 1. Schematic outline of the liposomal colchicine-derived prodrug strategy. PEGylated colchicine-derived prodrugs with different hydrolysis kinetics are synthesized by using different biodegradable PEG-linkers (A). These colchicinoid prodrugs are encapsulated into long-circulating liposomes (B). Upon hydrolysis of prodrug I or II in the aqueous core of the liposomes, the pharmacologically active parent is released from the liposomes with rate k_I and k_{II} ($k_I > k_{II}$), respectively (C).