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Active and passive tumor targeting of a novel poorly soluble cyclin dependent kinase inhibitor, JNJ-7706621

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ABSTRACT

The anti-cancer cyclin dependent kinase (CDK) inhibitors are poorly soluble drugs. The aims of this work were (i) to formulate a novel CDK inhibitor, JNJ-7706621, in polymeric micelles and nanoparticles, (ii) to compare passive and active targeting on tumor growth and (iii) to evaluate the potential synergy of JNJ-7706621 with Paclitaxel. Therefore, JNJ-7706621 was encapsulated in self-assembling diblock copolymers made up of ε-caprolactone (CL) and trimethylene carbonate (TMC) (PEG-p-(CL-*co*-TMC)) polymeric micelles and in (poly(lactide-co-glycolide)) (PLGA)-based PEGylated nanoparticles (passive targeting) as well as in RGD-grafted nanoparticles (active targeting). In vivo, the transplantable liver tumor growth was more decreased by active targeting with RGD-grafted nanoparticles than by passive targeting with micelles or ungrafted nanoparticles. Moreover, a synergy between JNJ-7706621 and Paclitaxel was demonstrated. Therefore, active targeting of JNJ-7706621-loaded nanocarriers may be considered as an effective anti-cancer drug delivery system for cancer chemotherapy, particularly in combination with Paclitaxel.

1. Introduction

Cyclin dependent kinases (CDKs) control cell growth by regulating the progression of cells through the cell cycle. This process is regulated by the coordinated action of CDKs in association with their specific regulatory cyclin proteins. Each step in the cell cycle is regulated by a distinct and specific CDK. The combination of catalytic kinase subunits (such as CDK1, CDK2, CDK4 or CDK6) with regulatory cyclin subunits (such as cyclin A, B, D1, D2, D3 or E) results in the assembly of functionally distinct kinase complexes (Emanuel et al., 2005). Over-expression of CDK1 and CDK4 has been reported in a large variety of tumors. Therefore, selective inhibition of CDKs may limit the progression of a tumor cell through the cell cycle and facilitate the induction of apoptotic pathways. Most current anti-mitotic anti-cancer drugs have undesirable side effects on cells. CDK inhibitors have the potential to arrest cell growth with fewer side effects and may also avoid the problem of multidrug resistance (Lapenna and Giordano, 2009; Zhang et al., 2009).

JNJ-7706621 (Fig. 1) is a 1,2,4-triazole-3,5-diamine derivative that inhibits CDK activity and is being developed as an anti-tumor agent. JNJ-7706621 is a potent inhibitor of CDK1, CDK2 and to lesser extent, CDK4 activity (Lin et al., 2005). JNJ-7706621 blocks progression of human cancer cells through the cell cycle causing an accumulation of cells in the G_2/M phase, preventing cells from entering mitosis and activating apoptosis. CDK inhibitors, including JNJ-7706621, are poorly soluble in aqueous medium (17 μg/ml) (Emanuel et al., 2005). Therefore, the current challenge is to formulate JNJ-7706621 for the intravenous route in a non-toxic vehicle.

The percentage of apoptotic cells as well as the degree of cytotoxicity induced by CDK inhibitors could significantly be increased by treatment of tumor cells with cytostatic drugs that are known to induce apoptosis of tumor cells in all phases of the cell cycle (Sedlacek, 2001; Schwartz et al., 2002). More particularly, flavopiridol, a CDK inhibitor developed by Sanofi-Aventis, has been shown to act in synergy with several cytostatics including Paclitaxel (PTX), offering an attractive clinical treatment opportunity. PTX is an effective anti-cancer drug active against a wide variety of tumors, including ovarian carcinoma, metastatic breast cancer, head and neck cancers and non-small lung cancer (Schiff and Horwitz, 1980). PTX is currently formulated (Taxol®) at the concentration of 6 mg/ml dissolved in mixture of Cremophor® EL (polyethoxylated caster oil) and ethanol (50:50, v/v) (Nicolaou et al., 2009).

Polymeric micelles (Fig. 2) are arranged in a spheroidal structure with hydrophobic core which increases the solubility of poorly water-soluble drugs, and the hydrophilic corona which allows for a long circulation time of the drug by preventing the interactions between the core and the blood components. These systems are dynamic and have a size usually below 50 nm (Kwon, 2002). Due to their prolonged circulation time, polymeric micelles are able

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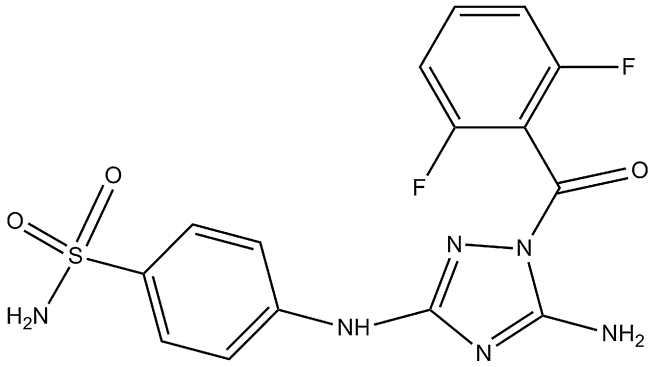

Fig. 1. Chemical structure of JNJ-7706621, 1-(2′,6′-difluorobenzoyl)-5-amino-3-(4′aminosulfonylanilino)-1,2,4-triazole.

to accumulate at certain biological sites characterized by vascular abnormalities, such as tumors, through the enhanced permeation and retention (EPR) effect (Maeda et al., 2009).

Nanoparticles (Fig. 2) are solid and spherical structures, ranging around 100 nm in size, in which drugs are encapsulated within the polymeric matrix (Byrne et al., 2008; Pirollo and Chang, 2008). Nanoparticles can also escape from the vasculature through the leaky endothelial tissue that surrounds the tumor and then accumulate in certain solid tumors by the EPR effect (Maeda et al., 2009). This phenomenon is called "*passive targeting*". The basis for increased tumor specificity is the differential accumulation of drug-loaded micelles or nanoparticles in tumor tissue versus normal tissues. Passive targeting can therefore result in increased drug concentrations in solid tumors of several-fold relative to those obtained with free drugs.

Target ligands attached to the surface of nanoparticles (Fig. 2) may act as "homing devices", improving the selective delivery of drug to specific tissue and cells (Pirollo and Chang, 2008). Among

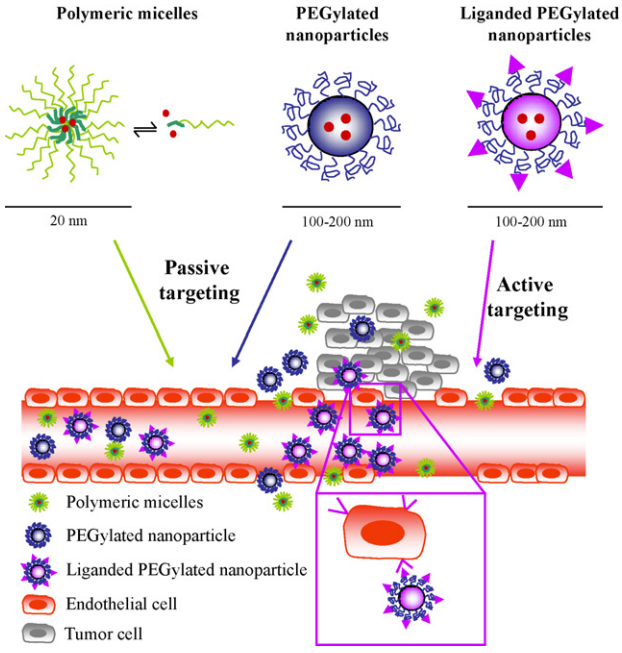

Fig. 2. Schematic representation of polymeric micelles, PEGylated nanoparticles and liganded nanoparticles. The PEGylation allows nanoparticles an extended circulation time, avoiding the opsonization of macrophages. Polymeric micelles and PEGylated nanoparticles reach tumors selectively through the leaky vasculature surrounding the tumors (EPR effect—passive targeting) whereas ligands grafted at the surface of nanoparticles allow active targeting by binding to receptors over-expressed by cancer cells or angiogenic endothelial cells.

various ligands currently developed allowing the "*active targeting*" of tumors, the tripeptide arginine-glycine-aspartic acid (RGD) has been shown to bind preferentially to integrins (in particular $\alpha_v\beta_3$) that are over-expressed in angiogenic endothelial cells. Targeting the $\alpha_v\beta_3$ integrin with drugs may provide an opportunity to target the tumor and to destroy tumor vessels without harmful effects on microvessels of normal tissue (Arap et al., 1998; Byrne et al., 2008).

As nanoencapsulation of poorly soluble CDK1 has never been studied for CDK solubilization and/or tumor targeting, the aims of this work were (i) to solubilize and formulate the novel CDK inhibitor, JNJ-7706621, for the parenteral route; (ii) to compare anti-cancer efficacy of this drug by passive or active targeting using polymeric micelles and nanoparticles; and finally (iii) to study the potential synergy of the JNJ-7706621 compound with PTX.

Therefore JNJ-7706621 was encapsulated in PEG-p-(CL-*co*-TMC) micelles and in PEGylated PLGA-based nanoparticles. Self-assembling diblock copolymers made up of ε-caprolactone (CL) and trimethylene carbonate (TMC) and mmePEG$_{750}$ (mmePEG$_{750}$-p-(CL-*co*-TMC)) have been shown to form micelles spontaneously upon gentle mixing with water. These copolymers are biocompatible, non-cytotoxic and non-hemolytic. They increase the solubility of poorly soluble-water drugs (including PTX) by one to four orders of magnitude (Ould-Ouali et al., 2004; Danhier et al., 2009a). PEGylated PLGA-based nanoparticles loaded with PTX prepared by nanoprecipitation method have been developed (Danhier et al., 2009b). Poly(lactide-co-glycolide) (PLGA) was chosen for its biodegradability properties, its biocompatibility and the fact that related polyesters have been previously approved by the FDA. Poly(ε-caprolactone-co-ethylene glycol) (PCL-PEG), an amphiphilic copolymer, was added to take advantage of PEG steric stabilization properties, to provide a higher stability of nanoparticles in biological fluids and to allow the grafting of RGD peptide on the polymer (Owens and Peppas, 2006; Garinot et al., 2007). RGD-grafted PLGA nanoparticles have been shown to target the $\alpha_v\beta_3$ integrin of the tumor endothelium (Danhier et al., 2009c). The in vitro anti-tumoral activity of JNJ-7706621-loaded micelles and nanoparticles was assessed using Human Cervix Epithelial Carcinoma (HeLa) cells. The apoptosis induced by JNJ-7706621-loaded micelles or nanoparticles was also studied. To compare the passive and active targeting of the anti-cancer drugs to tumors, in vivo anti-tumor efficacy of JNJ-7706621-loaded micelles, nanoparticles and RGD-grafted nanoparticles was investigated in mice bearing a fast growing tumor: the transplantable liver tumors (TLT). Finally, the combination of PTX-loaded micelles (Danhier et al., 2009a) or nanoparticles grafted or not (Danhier et al., 2009b,c) with these JNJ-7706621-loaded micelles or nanoparticles grafted or not was performed in TLT-tumor-bearing mice to evaluate the synergy between these two drugs.

2. Materials and methods

2.1. Materials

PLGA, PCL-b-PEG, PLGA-b-PEG polymers were synthesized by ring opening polymerization. Preparation of PCL-b-PEG grafted with GRGDS was performed as previously described by photografting (Garinot et al., 2007; Pourcelle et al., 2007). Molecular weights were determined by size exclusion chromatography (SEC) and NMR as described previously. The PEG-p(CL-*co*-TMC) diblock copolymers were synthesized by the Johnson and Johnson Advanced Technologies and Regenerative Medecine (ATRM) (Somerville, NJ, USA). Characteristics of polymers are summarized in Table 1. PTX was purchased from Calbiochem (Darmstadt, Germany). Taxol® was obtained from Brystol-Myers Squibb. JNJ-7706621 was provided by Johnson and Johnson, Pharmaceutical Research and Development (Beerse, Belgium).