

LIPID NANOPARTICLE MEDIATED DRUG DELIVERY FOR SAFER CANCER TREATMENT: EXAMPLE OF PACLITAXEL

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RESUMO

Os vectores coloidais de natureza lipídica têm atraído particular atenção para o desenvolvimento de terapias mais seguras e eficazes aplicadas a várias doenças. As vantagens da utilização das nanopartículas lipídicas são exemplificadas utilizando o paclitaxel, um anticancerígeno particularmente interessante para o desenvolvimento de novas formas farmacêuticas mais adequadas para o tratamento do cancro. De facto, este fármaco apresenta problemas de insolubilidade aquosa e sérios efeitos secundários concomitantes à administração da forma farmacêutica convencional (Taxol). Este artigo apresenta uma revisão das razões para a reformulação do paclitaxel e sumaria as vantagens de novas formas farmacêuticas contendo nanopartículas lipídicas para a administração deste anticancerígeno.

PALAVRAS-CHAVE

Nanopartículas de lípidos sólidos, vectores lipídicos nanoestruturados, nanopartículas lipídicas, paclitaxel, tratamento do cancro

ABSTRACT

Colloidal carriers composed of lipids attract much attention in the development of safer and more effective therapy of various diseases. The advantages of lipid-based nanoparticles are illustrated on the example of paclitaxel, a challenging chemotherapeutic drug for the development of a novel and more suitable dosage form for cancer treatment. Paclitaxel is known for its water insolubility and serious side effects when administered by its conventional formulation (Taxol). This paper reviews the reasons of further re-formulation of Paclitaxel and summarizes the achievements of lipid nanoparticle-based formulations of this drug.

KEYWORDS

Solid lipid nanoparticles, nanostructured lipid carriers, lipid nanoparticles, paclitaxel, cancer treatment

1. INTRODUCTION

Colloidal carriers composed of lipids have been developed to overcome some of the potential drawbacks of polymer-based colloidal drug carriers or liposomes. Since lipids are physiologically occurring compounds, they are well-tolerated, usually non-toxic and are degraded to non-toxic residues, which gives them an advantage over polymeric systems. Further on, solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) have shown superior stability in comparison to liposomes (Heurtault et al., 2003). SLN and NLC are versatile drug carriers suitable for delivery of wide range of drugs of both hydrophilic and lipophilic nature, and having different molecule weights.

Great attention is given to colloidal drug carriers in treatment of cancer. Various approaches in chemotherapeutic drug delivery has been reviewed recently, however, the reviews mostly focus on other types of colloidal carriers, such as liposomes, polymeric nanoparticles and micelles, dendrimers or inorganic nanoparticles. This paper summarizes the achievements of solid lipid nanoparticles, nanostructured lipid carriers and lipid nanocapsules in the delivery of a well known chemotherapeutic drug, paclitaxel.

2. MARKETING PACLITAXEL FORMULATIONS

Paclitaxel is a diterpenoid alkaloid drug approved by Food and Drug Administration (FDA) and European Medicines Agency (EMA) as first line therapy of breast and ovarian cancer, with proven effect against carcinomas of other tissues. Due to its physicochemical characteristics, formulating a suitable dosage form for its administration remains a challenging task.

Taxol (Bristol-Myers Squibb) was the first marketed formulation of Paclitaxel, introduced into market in 1992 as second-line treatment of advanced ovarian cancer. Although this formulation was successful in overcoming the water insolubility by employing a mixture of solubilizing agents, their use led to well known, and in literature extensively described, side effects. Briefly, polyoxyethoxylated castor oil (Cremophor EL) is pointed as the cause of severe hypersensitivity reactions upon administration; further on nephrotoxicity, neurotoxicity and hypotension have been reported. The potential risks associated with this formulation were summarized by Singla and colleagues (Singla et al., 2002).

Great efforts have been made to design a Cremophor EL-free formulation of paclitaxel. The aim is to find a biocompatible and well-tolerated material capable of solubilizing this drug without exposing the patient to additional risks. Various approaches have been used to design such a formulation.

The chemical structure of paclitaxel which does not contain enough ionizable groups practically limits the possibility to influence its solubility by pH changes. The chemical approach, focused on the drug molecule, is therefore in synthesizing prodrug forms that would show better solubility in water or at least in common pharmaceutical solvents. More recently, drug-polymer and drug-antibody conjugates have been designed and tested, mostly in *in-vitro* conditions. Some of the most significant outcomes of these studies were summarized recently by Safavy (Safavy, 2008).

Another approach is in search for better paclitaxel formulation. In fact, a highly hydrophobic compound is a challenge in terms of development of a dosage form. Combinations of co-solvents and oil-in-water emulsions have been tested by various research groups in the beginning of the nineties (Safavy, 2008).

With the development of colloidal drug carriers the possibilities of formulation of a water insoluble drug were increased. Liposomes technology was among the first tested. Although many liposomal formulations with sufficient stability have been developed, the clinical trials indicated no clear benefit over established formulation, and up-to-date there is no marketed liposomal formulation. Paclitaxel is also a suitable candidate for delivery by nanoparticles composed of wide array of materials. It was only with human serum albumin (HSA) based nanoparticles that another formulation of paclitaxel reached the market in 2005. As expected, the paclitaxel-loaded HAS based nanoparticles do not cause hypersensitivity reactions, which enables omitting the premedication and administration of higher doses in shorter times (Micha et al., 2006). However, it was shown that despite its small size, the drug shows similar body distribution as from Taxol (Henderson and Bhatia, 2007). The benefit of this formulation is indeed rather in use of biocompatible material to solubilize the drug than in colloidal size of its carriers.

Development of various colloidal carriers for paclitaxel delivery therefore still continues. The use of nanoparticles in general may help improving the delivery of drug into the target cells and thus increases the pharmacological effect. Moreover, controlled release and increased terminal half life can be achieved, which would subsequently allow less frequent administration. As great inter-individual differences in pharmacokinetics of paclitaxel have been observed after Taxol administration, also ascribed to Cremophor-EL (van Tellingen et al., 1999), the improvement of pharmacokinetic characteristics is also of high importance in development of novel paclitaxel formulations.

3. FUTURE PACLITAXEL FORMULATIONS?

3.1. NANOPARTICLE FORMULATIONS IN CLINICAL TRIALS

Several polymeric nanoparticle-based formulations are under clinical trials, formulations of paclitaxel not being an exception. In March 2009, FDA approved a Phase I clinical trial of Nanoxel, a polymeric nanoparticle formulation of paclitaxel developed by Fresenius Cabi. As great variety of biodegradable polymers-based nanoparticles and polymeric micelles have been reported in the literature, more nanoparticle formulations are expected to reach clinical trials soon.

In parallel with polymer-based drug delivery systems, those composed of lipid materials are being developed. This review will focus on lipid nanoparticle, such as SLN, NLC, and lipid nanocapsules (LNC), with proven suitability for paclitaxel loading.

3.2. LIPID NANOPARTICLES

Lipids and phospholipids have a great advantage of being identical or very similar to physiologically occurring compounds. Also, the use of lipids has a long tradition in pharmaceutical

formulations. Therefore, a majority of the lipids suitable for development of colloidal carriers have the generally regarded as safe (GRAS) status.

In the literature, many types of lipid nanoparticles have been reported. SLN are regarded particles with size under 1 μm composed of a lipid matrix that is solid at room and body temperature (Wissing et al., 2004). NLC are novel generation of lipid nanoparticles based on SLN, but consisting of a blend of a solid and liquid lipid, which creates a less crystalline yet solid lipid core. The advantage over SLN should be the possibility of higher drug loading and possibility of incorporating the drugs better soluble in liquid lipids into a colloidal carrier (Müller et al., 2002).

Not all formulations referred to as SLN in the literature in fact comply with the definition of solid lipid matrix. Instead of SLN, they better comply with the definition of lipid nanocapsules (LNC) - colloidal carriers composed of a liquid lipid core and a phospholipid shell, not necessarily solid (Huynh et al., in press). A wide variety of colloidal carriers composed of various lipids are also denominated as lipid nanoparticles, without further characteristics.

3.3. SLN FOR PACLITAXEL DELIVERY

The first studies on SLN for paclitaxel delivery were reported by Cavalli and Miglietta in 2000 (Cavalli et al., 2000, Miglietta et al., 2000). Cavalli et al developed tripalmitine-based SLN stabilised by soy phosphatidylcholine, with a mean particle size below 500 nm, with proven stability in isotonic glycerol solution. These nanoparticles could be freeze dried and reconstituted with only a small particle growth, and up to 2.8 % (wt) of paclitaxel could be encapsulated without precipitation of the drug during one year. The possible advantage towards clinical use already achieved in this study is the possibility of administration of usual therapeutic dose of 10 mg/m²/h in only 20-40 ml of SLN formulation with 2.0 % paclitaxel loading (Cavalli et al., 2000). Cytotoxicity of this paclitaxel SLN formulation was tested on MCF-7 (human breast carcinoma) and HL60 (human promyelocytic leukemia) cell lines. While drug free SLN showed no toxic effect towards these cell lines, paclitaxel loaded SLN caused growth inhibition of MCF-7 cells already in very low concentrations (1 ng/ml), at which the paclitaxel solution was not effective yet. Cell internalization of SLN of same lipid and surfactant composition loaded with 6-coumarine was confirmed by fluorescent microscopy (Miglietta et al., 2000). One year later, long circulating paclitaxel-loaded SLN with hydrophilic surface coating were reported with significantly higher half-time ($t_{1/2}$) and area under curve (AUC), in comparison with free paclitaxel (administered to mice as Taxol) (Chen et al., 2001).

Since the SLN showed clear advantages in the very first results, various research groups worldwide focused on development and further improvement of SLN formulations. A lipophilic drug should be an ideal candidate for lipid-based formulations. However, the solubility of paclitaxel in some of the solid lipids was reported to be low (Koziara et al., 2005, Videira et al., 2005), which does not enable obtaining sufficiently high drug loading. The simple and effective method to overcome this obstacle is dissolving the drug in ethanol and preparing the system either by solvent diffusion evaporation technique, by solvent injection or by via microemulsion method. In the course of SLN preparation, the solvent used to solubilize paclitaxel is then removed. Employing ethanol for improving paclitaxel solubility in solid lipids was first reported by Videira and colleagues (Videira et al., 2005) and it has been adopted by other research groups (Yuan et al., 2008, Zhang et al., 2008). It was showed for stearic acid-

based SLN that ethanol could be used (Yuan et al., 2008), instead of acetone used in one of the first reports about paclitaxel-loaded SLN (Chen et al., 2001). The method of solubilization of paclitaxel in ethanol enables formulating colloidal carrier for paclitaxel delivery based on practically any solid lipid or phospholipid. Table 1 gives the summary of lipid material used in SLN, NLC and lipid nanocapsules preparation in the up-to-date published studies.

With regard to lipid nanocapsules, good solubility of paclitaxel in medium chain triglycerides (a liquid mixture of caprylic and capric acid triglycerides), was reported (Babu Dhanikula et al., 2007); and in phospholipids with short saturated chains (Feng and Huang, 2001). This information led the research group of Dong to design a statistically optimized SLN formulation (Dong et al., 2009). Trilaurine-based SLN formulation stabilised by Polyoxyol 20-stearyl ether (Brij 78) and a lipid nanocapsules formulation comprising medium chain triglycerides (Miglyol 812), Polyoxyol 20-stearyl ether and D-alpha-tocopheryl polyethylene glycol 1000 succinate were the outcome of this statistical approach. Even though paclitaxel was added to the lipid phase of the formulation as ethanolic solution, and the solvent was immediately removed by nitrogen prior to proceeding to further steps of preparation. Moving towards pre-clinical studies, cytotoxicity of the developed formulations has been tested on different types of cell lines and in-vivo studies have been reported.

Table 1. Composition and focus of the studies on solid lipid nanoparticles for paclitaxel delivery

SLN Composition		Focus of the studies	Reference
Solid lipid	Surfactant(s)		
Trilaurine	Polyoxyol 20-stearyl ether	Phys. Chem. Char.; In-vitro evaluation; MDA-MB-231	(Dong et al., 2009)
Trimyristine	Egg Phosphatidylcholine + PEG-Phosphatidylethanolamine	Phys.Chem.Char.; In-vitro evaluation ; OVCA-3; MCF-7	(Lee et al., 2007)
Tripalmitine	Soybean Phosphatidylcholine + Butanol + Sodium taurocholate	Phys.Chem.Char	(Cavalli et al., 2000)
		In-vitro evaluation; MCF-7; HC60	(Miglietta et al., 2000)
		In-vitro evaluation HT-29	(Serpe et al., 2004)
Tristearin	Poloxamer 188	Phys.Chem.Char.; In-vitro evaluation A549	(Yuan et al., 2008)
Glycerol Behenate	Poloxamer 188	Phys.Chem.Char.; In-vitro evaluation A549	(Yuan et al. 2008)
Glycerol Palmito stearate		Phys.Chem.Char.; In-vitro evaluation B16F10	(Shenoy et al., 2009a)
Monostearin	Poloxamer 188	Phys.Chem.Char.; In-vitro evaluation A549	(Yuan et al. 2008)
	Poloxamer 407	Phys.Chem.Char.; In-vitro evaluation B16F10	(Shenoy et al., 2009b)
Monostearin + Oleic acid	Poloxamer 188	In-vitro evaluation; MCF-7; MCF-7/ADR; SKOV3; SKOV3-TR30	(Zhang et al., 2008)
Monostearin-PEG-SA, Monostearin-FA-SA	Poloxamer 188	Phys.Chem.Char.; In-vitro evaluation A549	(Yuan et al. 2008)
Stearic Acid	Polyoxyol 20-stearyl ether	PK - mice	(Chen et al., 2001)
	Poloxamer 188	PK - mice	(Chen et al. 2001)
		Phys.Chem.Char.; In-vitro evaluation A549	(Yuan et al. 2008)
	Poloxamer 188 + Phosphatidylcholine	Phys.Chem.Char.; In-vitro evaluation HepG2	(Pandita et al., 2009)
Cetyl alcohol + polysorbate 60	Polyoxyol 20-stearyl ether	In-vitro evaluation; U-118; HCT-15; BBB perfusion	(Koziara et al., 2004)
		BD (BALB/c mice); In-vitro evaluation; U-118; HCT-15	(Koziara et al., 2006)

Abbreviations: Phys.Chem.Char. = physicochemical characterization (zeta-potential, Scanning Electron Microscopy, Transmission electron microscopy, Differential scanning calorimetry, Wide-angle X-ray scattering, in-vitro release); PK = pharmacokinetic studies, BD = body distribution studies; BBB = blood-brain barrier

The first in-vitro study revealed much higher efficiency of paclitaxel-loaded SLN than Taxol in breast carcinoma cells. Subsequently, efficiency similar to Taxol have also been reported in human colorectal adenocarcinoma cell line (Serpe et al., 2004), human colorectal carcinoma resistant to paclitaxel and human glioblastoma cell line (Koziara et al., 2004). Under spe-

cial experimental conditions, paclitaxel encapsulated in SLN was found to be able to cross blood-brain barrier in rats, which was not possible with free paclitaxel (Koziara et al., 2004).

The group of Koziara and colleagues focused on cetyl palmitate-based SLN, which were shown to have minimal blood-toxicity (Koziara et al., 2005) and therefore suitable for intravenous administration. The formulation that showed excellent efficiency in growth inhibition in cell culture was also tested in mice with results significantly different from Taxol on the day 12 of the study. The body distribution of the paclitaxel also did not differ significantly from free drug (Koziara et al., 2006). The explanation suggested by this research team was the relatively fast release of the drug already in in-vitro experiments and its low loading in SLN, which stress the need to design a formulation which would entrap higher amount of drug and assure its controlled release. Moreover, this research group suggested that paclitaxel encapsulated in SLN could overcome the membrane transporter-mediated resistance to taxenes.

More in-vitro studies confirmed that SLN and NLC formulations could be effective even in taxenes-resistant cell lines. Zhang and colleagues reported similar inhibition concentrations (IC_{50}) of paclitaxel-loaded NLC required to cease the growth of both sensitive and resistant cancer cell lines, while 30 times higher concentration of Taxol was required to kill the resistant cells (Zhang et al., 2008). The authors also reported that folic acid-surfaced NLC were required in even lower concentrations to inhibit cancer cell growth.

Yuan and colleagues observed cellular uptake of paclitaxel-loaded SLN (here loaded also with fluorescein isothiocyanate) by confocal microscopy (Yuan et al., 2008). This study also gives interesting results of very low cytotoxicity of blank SLN formulation composed of various lipids (see Table 1), illustrated by IC_{50} of 308.72 to 471.48 $\mu\text{g ml}^{-1}$ in contrast to IC_{50} of 0.21 to 1.86 $\mu\text{g ml}^{-1}$ of paclitaxel-loaded SLN (which is still less than IC_{50} of Taxol).

3.4. LIPID NANOCAPSULES

Paclitaxel solubility in medium chain triglycerides or short-chain phospholipids makes it a perfect candidate for encapsulation in lipid nanocapsules (LNC). Indeed, stable lipid nanocapsules were developed by some research groups. LNC composed of Labrasol, soy phosphatidylcholine and polyethylene glycol-660 hydroxystearate with size below 100 nm were prepared and characterized by Lacoëuille and colleagues. These carriers showed a sustained release of paclitaxel over two weeks. During the release studies, LNC were reported as stable; nevertheless, long-term stability over this period is not reported (Lacoëuille et al., 2007a). In an in-vivo study conducted in Wistar rats, pharmacokinetic parameters and survival rates similar to Taxol were obtained (Lacoëuille et al., 2007b).

LNC composed of combination of Miglyol 812 with Brij 78 and D-alpha-tocopheryl polyethylene glycol 1000 succinate were developed by the same statistical analysis as trilaurine-composed SLN and proved efficient in inhibiting the growth of MDA-MB-231 cells (Dong et al., 2009).

Lipid nanoparticles for paclitaxel delivery composed by soy phosphatidylcholine and various sucrose fatty acid esters were designed and characterized (Arica Yegin et al., 2006). In-vitro testing of these nanoparticles showed prolonged release; in-vivo efficiency is still to be explored.

4. FURTHER PERSPECTIVES

All up-to-date published in-vitro studies compare effectiveness of lipid nanoparticles to that of Taxol. As a nanoparticle-bound formulation is already marketed, it would be of great interest to compare a lipid nanoparticle formulation to this protein-based marketed formulation. As one of the reasons of development of nanoparticulate formulations is to provide controlled release and protect the drug until it reaches its site of action – and this is doubtful in case of Abraxane (Henderson and Bhatia, 2007), the future nanoparticle-based formulation will need to fulfill these expectations.

Lipid nanoparticles are explored for a shorter period of time than liposomes, polymeric nanoparticles or micelles, yet some of their features make them more advantageous, namely in terms of biocompatibility and toxicity (degradation products including), and scaling up the manufacture process. If the lipid nanoparticle-based formulations are intended for intravenous administration, size of the particles needs to be maintained in suitable size range. Some of the authors proposed their nanoparticle formulations with larger diameters for oral administration, but the suitability for this administration route also needs to be proven by preclinical studies.

5. CONCLUSIONS

As the efforts to develop a safer and more efficient formulation for paclitaxel still continue, it is expected that more nanoparticle-based formulations will enter clinical trials soon. Based on the pre-clinical data obtained for SLN, NLC and LNC, these can be considered as suitable carriers for paclitaxel. In addition to advantages of lipid-based colloidal carriers in the general, these colloidal carriers might be promising for the future therapy of cancer.

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