International Journal of Pharmaceutics 379 (2009) 201–209

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Review

Lipid nanocapsules: A new platform for nanomedicine

N.T. Huynh, C. Passirani*, P. Saulnier, J.P. Benoit

Inserm U646, Université d'Angers, 10 rue André Boquel, F-49100 Angers, France

ARTICLE INFO

Article history:
Received 19 February 2009
Received in revised form 14 April 2009
Accepted 20 April 2009
Available online 3 May 2009

Keywords:
Nanocarriers
Nanotechnology
Anticancer drugs
Targeting

ABSTRACT

Nanomedicine, an emerging new field created by the fusion of nanotechnology and medicine, is one of the most promising pathways for the development of effective targeted therapies with oncology being the earlier and the most notable beneficiary to date. Indeed, drug-loaded nanoparticles provide an ideal solution to overcome the low selectivity of the anticancer drugs towards the cancer cells in regards to normal cells and the induced severe side-effects, thanks to their passive and/or active targeting to cancer tissues. Liposome-based systems encapsulating drugs are already used in some cancer therapies (e.g. Myocet, Daunoxome, Doxil). But liposomes have some important drawbacks: they have a low capacity to encapsulate lipophilic drugs (even though it exists), they are manufactured through processes involving organic solvents, and they are leaky, unstable in biological fluids and more generally in aqueous solutions for being commercialized as such. We have developed new nano-cargos, the lipid nanocapsules, with sizes below the endothelium fenestration (ϕ < 100 nm), that solve these disadvantages. They are prepared according to a solvent-free process and they are stable for at least one year in suspension ready for injection, which should reduce considerably the cost and convenience for treatment. Moreover, these new nano-cargos have the ability to encapsulate efficiently lipophilic drugs, offering a pharmaceutical solution for their intravenous administration.

The lipid nanocapsules (LNCs) have been prepared according to an original method based on a phase-inversion temperature process recently developed and patented. Their structure is a hybrid between polymeric nanocapsules and liposomes because of their oily core which is surrounded by a tensioactive rigid membrane. They have a lipoprotein-like structure. Their size can be adjusted below 100 nm with a narrow distribution. Importantly, these properties confer great stability to the structure (physical stability > 18 months). Blank or drug-loaded LNCs can be prepared, with or without PEG (polyethyleneglycol)ylation that is a key parameter that affects the vascular residence time of the nano-cargos. Other hydrophilic tails can also be grafted. Different anticancer drugs (paclitaxel, docetaxel, etoposide, hydroxytamoxifen, doxorubicin, etc.) have been encapsulated. They all are released according to a sustained pattern. Preclinical studies on cell cultures and animal models of tumors have been performed, showing promising results.

Contents

* Corresponding author. Tel.: +33 241 735850; fax: +33 241 735853.
E-mail address: catherine.passirani@univ-angers.fr (C. Passirani).

0378-5173/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2009.04.026

1. Introduction

Research and development (R&D) of new chemical or biological entities applied in therapeutics is a multimodal field requiring continuous commitment to conduct worldwide, scientific research. Globally, pharmaceutical R&D expenditure has increased continually year by year. However, according to data from the FDA (Food and Drug Administration), the number of new molecular entities (NMEs) approved by the FDA is decreasing. 53 NMEs received FDA approval in 1996, 27 in 2000, and only 22 in 2006. At the same time, major patents for blockbuster drugs will arrive at the end of their validity over the next few years, and the pharmaceutical industry is currently exploring a drug strategy to reposition existing active ingredients. One striking example is the case of sildenafil (VIAGRA®), the first selective-type 5-phosphodiesterase inhibitor. It was initially studied for use in angina pectoris. Interestingly, the results of the first clinical trial suggested that the drug had little effect on angina, but that it could induce marked penile erections (Osterloh and Riley, 2002; Ghofrani et al., 2006) so that it has now become an effective 'on demand' treatment of erectile dysfunction in men. Thus, this "repositioning" strategy leading to the discovery of new indications, new targets and also new drug delivery systems constitutes the foundation of nanomedicine which has attracted considerable interest in the recent years as an emerging new field created by the fusion of nanotechnology and medicine. Indeed, it can provide medical and pharmaceutical benefits, especially in oncology, because it enables the control of drug characteristics such as solubility, vascular circulation time, and specific site-targeted delivery (Caruthers et al., 2007). Thanks to their passive and/or active targeting of cancer tissue, drug-loaded nanoparticles provide an ideal solution, leading to selective cytotoxicity in the targeted tumor cells while also preventing harm to healthy cells. In this way, many anticancer agents have been incorporated into nanoparticles and have so far gained some success in research in the field of therapeutic application (Working and Dayan, 1996; Steiniger et al., 2004; Haley and Frenkel, 2008).

Taking this "repositioning" strategy in consideration, a number of colloidal drug delivery systems with a size below 100 nm have been designed to encapsulate the drug in carriers including micelles, different types of liposomes, nanoparticles made from polymers or lipids, polymer–drug conjugates, dendrimers, ... (Letchford and Burt, 2007). Among these carriers, our research group has recently developed and patented biomimetic carriers that mimic lipoproteins: lipid nanocapsules (LNCs) (Table 1) (Heurtault et al., 2000). Their size ranges from 20 to 100 nm, and they are characterized by a hybrid structure between polymer nanocapsules and liposomes. As compared to liposomes which manufacture through processes involving organic solvent and are leaky, unstable in biological fluids, LNCs are prepared by a solvent-free, soft-energy procedure and present a great stability (with physical stability up to 18 months). They have generally an oily core, corresponding to medium-chain triglycerides surrounded by a membrane made from a mixture of lecithin and a pegylated surfactant. Their formulation is based on the phase-inversion temperature phenomenon of an emulsion leading to lipid nanocapsule formation with good mono-dispersion (Heurtault et al., 2002a).

In this review, we will focus on LNCs with a description of their preparation process and their physical characteristics. We will also discuss the various strategies used in drug delivery for cancer treatment using LNCs as potential targeting carriers.

2. LNC formulation

2.1. Preparation

LNC formulation is based on at least three principal components: an oily phase, an aqueous phase and a nonionic surfactant. The oily phase is essentially constituted of triglycerides of capric and caprylic acids known under the commercial name of Labrafac® WR 1349. The hydrophilic surfactant, Solutol® HS 15, is derived from polyethyleneglycol (PEG) and is a mixture of free PEG 660 and PEG 660 hydroxystearate. The aqueous phase consists of MiliQ® water plus sodium chloride salt, NaCl. Furthermore, another surfactant, Lipoid®, composed of 69% phosphatidylcholine soya bean lecithin, is used in small proportions to significantly increase LNC stability (Minkov et al., 2005; Vonarbourg et al., 2005), which is especially necessary in the case of 50–100 nm LNC formulations. All components are approved by the FDA for oral, topical and parenteral administration. Each component has different influences on LNC formulation and stability which are cited in Table 2.

According to the patent No. WO02688000 (Heurtault et al., 2000), the preparation process of this type of lipid nanocapsule involves two steps. Step I consists in mixing all the components (whose proportions vary according to the study) under magnetic stirring and heating from room temperature up to T2 temperature, above the phase-inversion temperature (PIT), to obtain a W/O emulsion. This is followed by a cooling process to the T1 temperature, below the PIT, leading to the formation of an O/W emulsion. Several temperature cycles crossing the phase-inversion zone (PIZ) between T2 and T1 are then carried out. The temperature before

Table 1
Patents related to LNC formulation.

Patent	Authors	Number
Lipidic nanocapsules: preparation process and use as drug delivery systems	Saulnier, P., Heurtault, B., Benoit, J.P., Proust, J.E., Pech, B., Richard, J.	WO02688000
Nanocapsules with liquid lipidic core loaded with water-soluble or water-dispersible ingredient(s)	Anton, N., Saulnier, P., Benoit, J.P.	WO2009001019
Method for preparing lipid nanoparticles	Benoit, J.P., Anton, N., Saulnier, P.	WO2009004214
Aqueous core lipidic nanocapsule encapsulation and release of fragile hydrophilic et/ou lipophilic drugs	Anton, N., Saulnier, P., Benoit, J.P.	PCT/EP2008/062435
Nanocapsules of lipophilic complexes of nucleic acids	Saulnier, P., Benoit, J.P., Passirani, C., Vonarbourg, A., Lambert, O., Pitard, B.	WO2008096321

Table 2
Factors influencing the formulation and the stability of LNC prepared by phase-inversion temperature (PIT) method.

Factors	Effects	Reference
Nonionic surfactant amount (Solutol®)	Major influence on LNC formation and stability	Heurtault et al. (2002b, 2003c), Anton et al. (2007a)
Temperature cycles	Favoring LNC formation and improving the quality of LNC dispersion	Anton et al. (2007a, 2008)
Oil proportions (Labrafac®)	Increase of LNC size	Heurtault et al. (2003c)
NaCl	Decrease of PIT	Heurtault et al. (2002a), Anton et al. (2007b)
Lipophile surfactant (Lipoid®)	Stabilizing the LNC rigid shell and favoring the freeze-drying process	Dulieu and Bazile (2005), Vonarbourg et al. (2005)

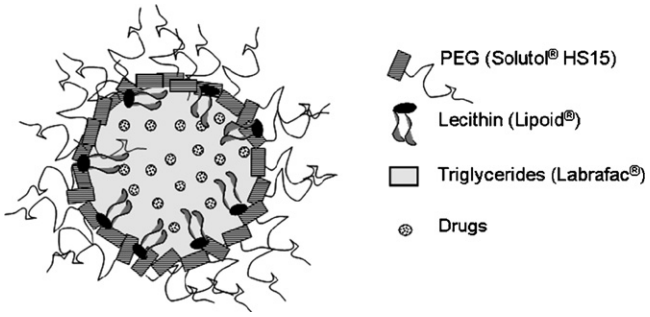

Fig. 1. Schematic representation of LNC prepared by the phase-inversion temperature method.

dilution is determined at the beginning of the inversion process and is defined by a temperature range that is set at 1–3 °C from the beginning of the O/W emulsion. Step II is an irreversible shock, induced by sudden dilution with cold water added to the mixture which has been maintained at the previously defined temperature. This is done to break the microemulsion system obtained in the PIZ, and leads to the formation of stable nanocapsules. Afterwards, slow magnetic stirring is applied to the suspension for 5 min. Different composition proportions are prepared in order to explore the phase diagram (Heurtault et al., 2003c). Three temperature cycles of heating and cooling at the rate of 4 °C/min are usually applied between 85 and 60 °C (Heurtault et al., 2002a; Saulnier et al., 2008).

The above-mentioned process leads to the formation of lipid nanocapsules constituted of an oily core, corresponding to free Labrafac®. The tensioactive, cohesive membrane is made up of the mixture of Lipoid® anchored in the oily phase, and Solutol® oriented towards the water phase (Fig. 1) (Heurtault et al., 2003a).

2.2. PIT process—technological principles

The PIT method was first introduced by Shinoda and Saito (1969) and is now widely used in industry. PIT is defined as the "temperature or temperature range at which the hydrophilic and lipophilic properties of a nonionic surfactant just balance" (Friberg et al., 1976). This method is essentially based on the changes in solubility of polyoxyethylene-type, nonionic surfactants according to the temperature. These types of surfactant become lipophilic with increasing temperature as a consequence of the dehydration of polyoxyethylene chains due to the breakdown of hydrogen bonds with water molecules. At low temperatures, the surfactant monolayer has a large, positive, spontaneous curvature forming O/W emulsions, characterized by high conductivity values (35 mS/cm). By increasing the temperature, the spontaneous curvature becomes negative, leading to W/O emulsion formation as a rapid decrease in conductivity of nearly 0 mS/cm at 84 °C is observed. This suggests a phase-inversion process from an O/W to a W/O emulsion which takes place in the PIZ. This zone demonstrates a continuous variation of conductivity between W/O and O/W emulsions attributed to bicontinuous microemulsion structures in which the spontaneous curvature becomes close to zero (Morales et al., 2003; Solans et al., 2005).

The PIZ temperatures are strongly affected by salinity levels. Generally, the more sodium chloride added, the higher the conductivity (Miller et al., 2001). This directly influences the temperature of the first rise in conductivity (T_{rise}) during the cooling of the W/O emulsion, by modifying the solubility of the polyethoxylated surfactant (Anton et al., 2007b). T_{rise} decreases as the NaCl concentration increases, but the PIZ remains virtually unchanged. This provides the possibility to modify the temperature range, with salinity being of prime importance for the encapsulation of thermolabile drugs (Heurtault et al., 2002a). Furthermore, drug degradation is expected to be limited because of the short heating period. Indeed, an *in vitro* study on the effect of etoposide-loaded LNCs (Lamprecht and Benoit, 2006), a drug which possesses poor aqueous solubility and chemical instability (Shah et al., 1989), on glioma cell growth demonstrated that this technique preserved not only the integrity but also the activity of the drug.

2.3. Nanoemulsions and nanocapsules

Nanoemulsions are nanometric-scale emulsions, typically displaying droplet diameters in the range of 20–200 nm (Solans et al., 2005). In contrast to microemulsions that are thermodynamically stable systems that form spontaneously, nanoemulsions are only stable kinetically. Two fundamental processes may be applied for the preparation of nanoemulsions, either by high-energy emulsification methods (e.g. high pressure homogenizers (Floury et al., 2003) or ultrasound generators (Abismail et al., 1999)) or by low-energy methods (e.g. spontaneous emulsification (Bouchemal et al., 2004) or the phase-inversion temperature (PIT) process (Anton et al., 2007a)).

Both nanoemulsions and nanocapsules can be prepared by the PIT technique, generating some objects in the nanoscale range. To discriminate one from another, it is useful to review the ternary diagram consisting of different proportions of aqueous phase, oil phase and nonionic surfactant, and also the prismatic diagram obtained by adding the temperature parameter (Fig. 2a). The characterization of an entire prism may become complicated. However, this can be simplified by keeping constant either the concentration of nonionic surfactant, corresponding to χ in the diagram, or the water/oil ratio (WOR), corresponding to γ in the diagram. The latter represents the Fish-cut diagram which allows the Winsor system to be defined under equilibrium conditions (Winsor, 1954). As far as the temperature (T) axis is concerned, at low T levels, over the Winsor I region, O/W emulsions are formed; at higher T levels, the Winsor II region corresponds to the formation of W/O emulsions; at the intermediate T or PIT, the system is balanced; in this situation, the Winsor III region (consisting of water, microemulsion, and oil phases in equilibrium) or the Winsor IV region (single phase microemulsion) occurs (Fig. 2b) (Morales et al., 2003). Depending on the amount of surfactant, phase equilibrium may be found either in the Winsor III or in the Winsor IV region, generating different final formulations. With small amounts of nonionic surfactant, the three-phase region is observed. Near the PIT, this system has the minimum degree of interfacial tension. A sudden dilution in cold water of this system produces nanoemulsions. Conversely, with larger quantities of surfactant (>10 wt.%), when combined with a temperature

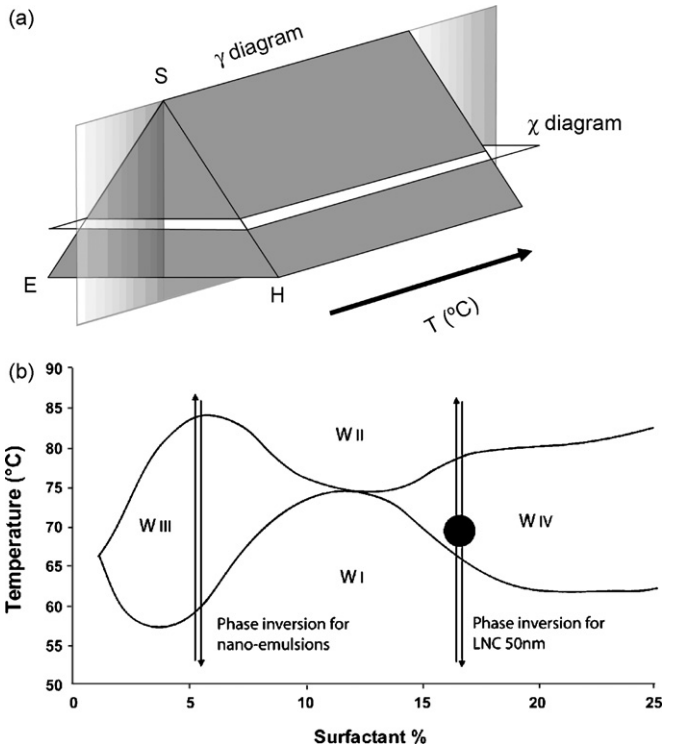

Fig. 2. Prismatic diagram (a) representing the correlation among proportions of aqueous phase, oil phase, nonionic surfactant amount and temperatures. At a constant water/oil ratio, the γ diagram or Fish-cut diagram (b) is obtained and allows determining the Winsor system under equilibrium conditions in the function of temperature and of surfactant amount.

cycling process, the rapid cooling of the Winsor IV system leads to the formation of lipid nanocapsules (Anton et al., 2008).

Since the temperature of this final formulation is below the nonionic surfactant melting point (about 30 °C), shell crystallization can occur; this prevents from the coalescence of the droplets and leads to the formulation of stable LNC suspensions at room temperature (Dulieu and Bazile, 2005). Thanks to this rigid shell, such suspensions allow freeze-drying to take place by means of adding a cryoprotectant, such as mannitol, glucose or trehalose. Among the tested cryoprotectants, trehalose provides the best polydispersity index of the sample after freeze-drying and resuspension (Heurtault et al., 2002a; Dulieu and Bazile, 2005). On the contrary, nanoemulsions are only stable kinetically, and one of the main instability problems of nanoemulsions involves the increase of the Ostwald ripening rate under storage. Ostwald ripening results from the difference in interfacial tension between small and large droplets, causing the diffusion of oil molecules from small to larger droplets (Tadros et al., 2004).

2.4. Physical characteristics

LNC particle size and dispersity are strongly dependent on the proportions of the constituents. A ternary diagram was established to optimize the constituent proportions before the cooling dilution takes place (Heurtault et al., 2002a, 2003c). By fixing the proportions of NaCl in water at 1.75% and of Lipoid® at 1.5%, a feasibility domain was determined as a parallelogram whose relative proportions were comprised approximately from 10 to 40% of hydrophilic surfactant, 35 to 80% of water, and 10 to 25% of oil (Fig. 3). Once nanoparticles are formed, their average volume size ranges from 20 to 100 nm, and exhibit a very narrow range of dispersity (PdI < 0.3).

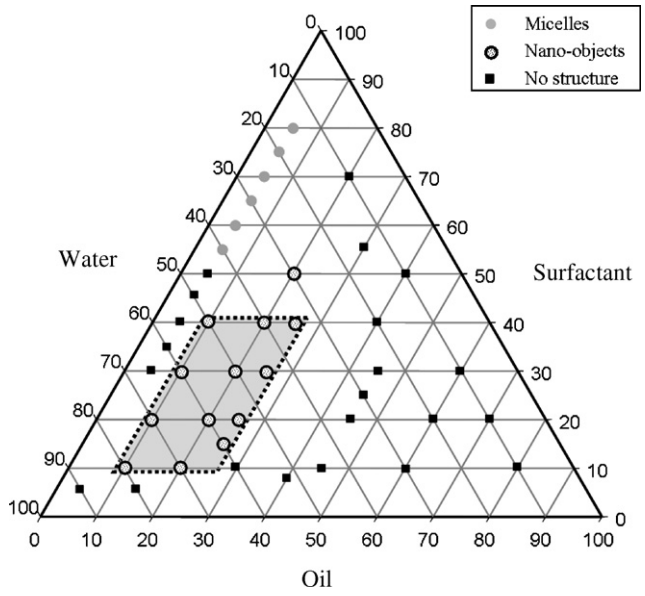

Fig. 3. The feasibility zone for the formation of LNC corresponding 10–40% of hydrophilic surfactant, 35–80% of water and 10–25% of oil by fixing the amount of NaCl at 1.75% and of lipophilic surfactant (Lipoid®) at 1.5% (inspired from (Heurtault et al., 2003c)).

In the region of feasibility, the percentage of hydrophilic surfactant (Solutol®) has a major effect on the average LNC diameters because, if it is increased, it leads to a considerable decrease of average particle diameter. This phenomenon results from its properties at the triglyceride/water interface (Heurtault et al., 2002b). Conversely, an increase of oil proportion leads to an increase of particle size whereas the proportion of water has no effect on particle diameter.

Furthermore, the temperature cycling process crossing the PIZ plays a relatively important role on LNC formulation. An increasing number of cycles favors LNC formation and improves the quality of LNC size and dispersion. Concretely, the less the amount of surfactant added, the higher the number of temperature cycles required to stabilize nanometric dispersion. For the larger amounts of surfactant, several cycles do not really appear to be necessary (Anton et al., 2007a). Within the feasibility zone, an increase of the number of temperature cycles to over 3 does not really seem to be beneficial to LNC size and polydispersity index reduction.

The zeta potential represents the electric potential at the LNC shear plane. This is an important and useful indicator to predict and control LNC stability (Heurtault et al., 2003b). The measurement of zeta potential is performed by means of a laser Doppler method. In general, LNCs have a negative surface charge due to the negative contribution of phospholipids molecules (Manconi et al., 2003) and the presence of PEG dipoles in their shell (Vonarbourg et al., 2005).

3. LNC development strategies

3.1. Drug-loading into LNCs and their release profiles

The existence of such nanoparticulate formulations provides an opportunity to encapsulate various kinds of molecules designed for anti-infection or anticancer action using various strategies for drug delivery to the tumor. Moreover, these nano-cargos present an efficient drug-loading mechanism with the encapsulation rates over 90% (Table 3). This encapsulation yield is much higher than that of liposomes (about 50%) (Wehrlé, 2007).

Firstly, amiodarone, an antiarrhythmic drug used in heart disease, was studied as a model drug encapsulated into LNCs (Lamprecht et al., 2002). Amiodarone was attracting attention

Table 3
Various strategies for drug delivery to the sites of action using LNC.

Strategies	Examples	Encapsulated drugs	Encapsulation rates	Study designs	Results	Reference
P-gp inhibition	LNC coated with PEG-type nonionic surfactants such as Solutol®	Etoposide	89.9 ± 2.3%	*In vitro* on C6, F98, 9L glioma cell lines	Increase cytotoxicity on glioma cells due to high intracellular drug accumulation	Lamprecht and Benoit (2006)
		Paclitaxel	93.0 ± 3.1%	*In vitro* on 9L and F98 glioma cell lines *In vivo* on s.c. F98 tumor model, single i.t. treatment at Day 5	Significant reduction in cell survival Significant reduction in tumor mass and tumor volume evolution	Garcion et al. (2006)
Passive targeting	Post-insertion of longer PEG chains: DSPE-PEG 1500; DSPE-PEG 2000; DSPE-PEG-5000	Drug-free		Biodistribution after an i.v. injection into healthy rats	Half-life time over 5h *vs* under 21 min for conventional LNC	Hoarau et al. (2004), Ballot et al. (2006), Beduneau et al. (2006)
	Post-insertion of DSPE-PEG 2000	Docetaxel	>98%	C26 colon adenocarcinoma s.c. tumor, i.v. injection of treatments in mice	Significant and substantial accumulation in the tumor *vs* conventional LNC and control docetaxel formulation (Taxotere®)	Khalid et al. (2006)
Active targeting	Attachment of OX26 Mab or Fab′ fragments at the LNC surface directed against TfR	Drug-free		*In vitro* cell binding on Y3.AG.1.2.3. cells and rat BCECs	Effective binding of immuno-nanocapsules on the cells *via* TfR	Beduneau et al. (2007a,b)
				Biodistribution after an i.v. injection into healthy rats	Significant accumulation in the brain 24h after administration *vs* non-targeted LNC	
Local administration (CED)	CED technique for delivery of LNC into the brain	^{188}Re-SSS; Fc-diOH	>98%	9L rat brain tumor intracranial xenograft model, CED treatment	Significant improvement in median survival time	Allard et al. (2008a)
Oral administration	LNC formulation to inhibit P-gp on the gastrointestinal tract	Paclitaxel	99.9 ± 1%	Oral administration by gastric intubation into healthy rats	Augmentation of mean plasmatic concentration of paclitaxel	Peltier et al. (2006)

P-gp: P-glycoprotein; LNC: lipid nanocapsules; PEG: polyethyleneglycol; s.c.: subcutaneous; i.t.: intratumoral; i.v.: intravenous; Mab: monoclonal antibodies; TfR: transferrin receptor; BCECs: brain cerebral endothelial cells; CED: convection-enhanced delivery; ^{188}Re-SSS: $^{188}Re(S_3CPh)_2(S_2CPh)$ complex; Fc-diOH: ferrocifenol.

because of its remarkable efficacy, combined with a long half-life, but was also accompanied by severe side-effects due to ocular, dermatologic, gastrointestinal, neurological, cardiovascular, thyroid, and pulmonary toxicity (Naccarelli et al., 1985). Nanocapsules of different sizes, loaded with amiodarone in their oily phase were analysed, mainly in terms of drug-loading and *in vitro* release profiles, and were compared to PLGA polymer nanoparticles. Interestingly, with the same drug content, the PLGA nanoparticles quickly released amiodarone, with a marked burst effect, whereas the LNCs showed only a small initial burst effect followed by slow sustained release of the encapsulated drug (Fig. 4). This controlled release might essentially result from lipophilic and amphiphilic properties that allow amiodarone to settle at the oil–water interface of formulated LNCs. This can be of interest in the field of drug delivery for topical or systemic application, and also in the domain of cosmetics.

Consequently, many lipophilic drugs, as well as amphiphilic drugs, have been prepared in LNC form as, for example, ibuprofen, incorporated into LNCs for pain treatment by intravenous administration (Lamprecht et al., 2004); indinavir, an inhibitor of HIV-1 protease (Pereira de Oliveira et al., 2005); various hydrophobic anticancer agents: etoposide (Lamprecht and Benoit, 2006), paclitaxel (Lacoeuille et al., 2007), tripentone (Malzert-Freon et al., 2006), derivatives of 4-hydroxy tamoxifen combined with ferrocen (Allard et al., 2008b), etc. In addition, LNCs can be loaded with different radionuclides in their oily core or at their surface such as ^{99m}Tc, ^{188}Re, ^{125}I or ^{111}In (Ballot et al., 2006; Jestin et al., 2007; Allard et al., 2008a). These LNCs provide opportunities for their application in imaging and radiotherapy.

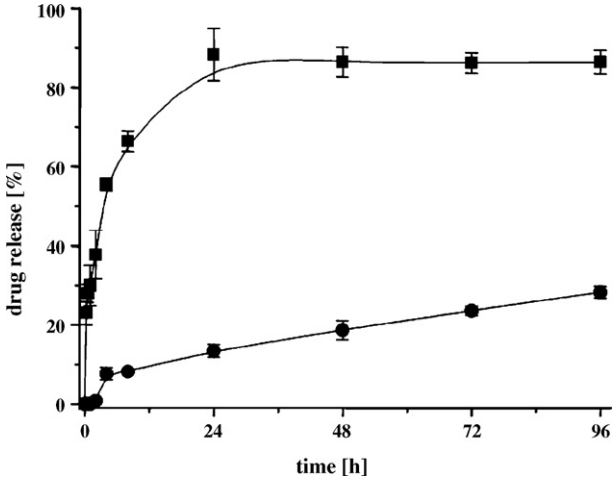

Fig. 4. *In vitro* release profile of amiodarone loaded LNC (circles) and PLGA-NP with blank LNC (squares) as acceptor phase in the release medium (inspired from (Lamprecht et al., 2002)).

3.2. P-glycoprotein inhibition

P-glycoprotein (P-gp), an ATP-dependent drug efflux pump, plays the role of a transporter of various lipophilic and cationic drugs/substrates, preventing sufficient accumulation of anticancer drugs within cells. Indeed, this is one of the main factors leading to the resistance of tumor cells to many anticancer agents

such as anthracyclines (doxorubicin, daunorubicin and epirubicin), *Vinca* alkaloids (vinblastine and vincristine), epipodophyllotoxins (etoposide and teniposide) and taxanes (paclitaxel and docetaxel) (Sparreboom and Nooter, 2000). Therefore, various strategies have been launched in scientific research in order to circumvent this problem (Modok et al., 2006). In fact, several experiments towards the inhibition of P-gp using nanoscale carrier-systems have been carried out. LNCs prepared by the phase-inversion process as described above, demonstrate P-gp inhibiting properties thanks to their ingredients, especially Solutol®. This surfactant is able to block P-gp-related drug efflux with a very low level of *in vitro* toxicity (Coon et al., 1991). Consequently, etoposide-loaded LNCs demonstrate simultaneously P-gp inhibition and sustained drug release from the LNCs (Lamprecht and Benoit, 2006). These adjuvant effects lead to a high level of intracellular, drug accumulation, resulting in increased *in vitro* cytotoxicity on glioma cells.

In this way, the inhibitory effect of multidrug resistance was investigated on an *in vitro* and *in vivo* glioma rat model, treated with either blank LNCs or paclitaxel-loaded LNCs (Garcion et al., 2006). After incorporation into LNCs, paclitaxel showed a potent reduction in 9L and F98 cell survival as compared to a free drug (Taxol®), whereas no significant difference was observed between these two formulations on newborn rat astrocyte primary cells that do not divide. In addition, on a subcutaneous F98 glioma model, paclitaxel-loaded LNC treatment by a single intratumoral injection, significantly reduced tumor mass as well as the evolution of tumor volume (Garcion et al., 2006).

3.3. Passive targeting

Colloidal drug carriers are rapidly removed from systemic circulation after intravenous injection due to their recognition as foreign bodies by the mononuclear phagocyte system (MPS), especially by Kupffer cells in the liver, macrophages in the spleen, and bone-marrow (Moghimi et al., 2001). This recognition is enhanced by the opsonization of the complement system by plasma proteins (Passirani and Benoit, 2005). The elimination of such colloidal systems is influenced by various parameters such as: the nature of the components, their size, the apparent electrical charge, their hydrophilicity (Vonarbourg et al., 2006a). Preferably, they have to be small, composed of natural compounds, and present a neutral and hydrophilic surface. A generation of nanoparticles coated with hydrophilic polymer chains, such as PEG and its derivatives, have been investigated for their property of prolonged circulation time in the bloodstream thanks to steric repulsion generated by the PEG layer (Mosqueira et al., 2001a; Moghimi and Szebeni, 2003).

LNCs appear to satisfy these critical properties because of their nanoscale size range and their shell consisting of a PEG 660 surfactant at high density. ^{188}Re/^{99m}Tc-labelled LNCs exhibited a blood half-life of 21 ± 1 min for ^{99m}Tc and 22 ± 2 min for ^{188}Re in rats (Ballot et al., 2006). Moreover, grafting longer PEG chains, such as PEG 1500 stearate instead of PEG 660 stearate, to the LNC surface provided an opportunity to prolong their circulation residence time (Beduneau et al., 2006). Consequently, the plasma elimination half-life time of these PEG 1500 stearate LNCs has been observed at around 5.5 h, with 20% of total dose still present in the blood 24 h after an intravenous injection into healthy rats. Thus, as well as the high density of PEG at the LNC surface, PEG flexibility linked to the curvature radius and PEG length also play an important role on macrophage uptake (Mosqueira et al., 2001b). These parameters, especially the length of PEG chains, can explain the rapid elimination of conventional LNCs from the blood circulation, despite their very weak complement activation (Ballot et al., 2006; Vonarbourg et al., 2006b).

Furthermore, it has been found to be possible to retain LNCs longer in systemic circulation by post-inserting distearoylphosphatidylethanolamine (DSPE)-PEG 2000 or DSPE-PEG 5000 at their surface with half-life times of over 6 h after intravenous administration (Hoarau et al., 2004). Thus, these pegylated nanocapsules are considered to be potential carriers for drug delivery to the sites of action, particularly into solid tumors due to enhanced permeability and the retention (EPR) effect (Maeda et al., 2000). Indeed, as a consequence of fast growth, tumor vessels are leaky while normal capillaries are found as tight junctions. This vascular defect, coupled with impaired lymphatic drainage, serves to enhance the permeability and retention of nanoparticles within the tumor region, and allow passive targeting to solid tumors using nanocarriers. As expected, docetaxel-loaded LNCs coated with DSPE-PEG 2000 significantly and substantially accumulated in C26 colon adenocarcinoma subcutaneous tumors, whereas uncoated LNCs showed poor tumoral accumulation (Khalid et al., 2006). Indeed, tumoral docetaxel concentrations increased over a 12 h sampling period and were much higher than those of a control docetaxel formulation (Taxotere®).

3.4. Active targeting

Whereas passive targeting takes advantage of a natural, physiological uptake mechanism, active targeting involves the attachment of a homing moiety, such as a monoclonal antibody (MAb) or a ligand, in order to deliver a drug to pathological sites or to cross biological barriers based on molecular recognition processes (Fenart et al., 1999; Brigger et al., 2002; Jallouli et al., 2007). This strategy appears useful for drug delivery into the brain which is limited mainly by the obstacle of the blood–brain barrier (BBB), separating the blood from the cerebral parenchyma (Pardridge, 2005). Consequently, active brain-targeting strategies that have been established to date, consist in grafting a MAb onto a nanocarrier, which can then recognise an over-expressed receptor on brain capillary endothelial cells (Beduneau et al., 2007a). In fact, several studies have investigated the use of OX26 monoclonal antibodies (OX26 MAb) as a vector for drug delivery across the BBB via a receptor-mediated transcytosis mechanism (Friden et al., 1991; Pardridge et al., 1991). This antibody can attach itself directly to the transferrin receptor (TfR) which is selectively localized on the brain capillary endothelium, and is found at low concentrations on other tissues (Jefferies et al., 1984). Moreover, TfR is over-expressed at the surface of proliferating cells such as brain tumor cells, especially on glioblastoma multiforme (Hall, 1991).

In this context, immuno-nanocapsules have been designed, by the conjugation of LNCs to whole OX26 MAb, for the purpose of actively transporting drugs to the brain parenchyma (Beduneau et al., 2007b). Furthermore, Fab′ fragments conjugated to LNCs have also been evaluated because of the interest of their reduced MPS uptake via the Fc receptor-mediated mechanism, which allows prolonged systemic circulation (Maruyama et al., 1997). This coupling has been facilitated by the incorporation of lipid PEG 2000, functionalized with reactive maleimide groups (DSPE–PEG 2000–maleimide), into LNC shells by a post-insertion procedure allowing the covalent attachment of the ligands to LNCs. The OX26 MAb and Fab′ elements were previously thiolated to enable them to react with the reactive maleimide group via thioether bonds. Except in cases where large amounts of antibodies are grafted onto the LNCs, in which case the sizes become heterogeneous, the obtained immuno-nanocapsule size ranged around 150 nm in diameter with a coupling efficiency comprised between 20 and 29%. On the other hand, a size increase after ligand conjugation moved the location of OX26 MAb and Fab′ to outside the PEG brush which facilitated cell association. Further research has included *in vitro* studies on cells over-expressing TfR, such as the Y3.AG.1.2.3. hybridoma cell line

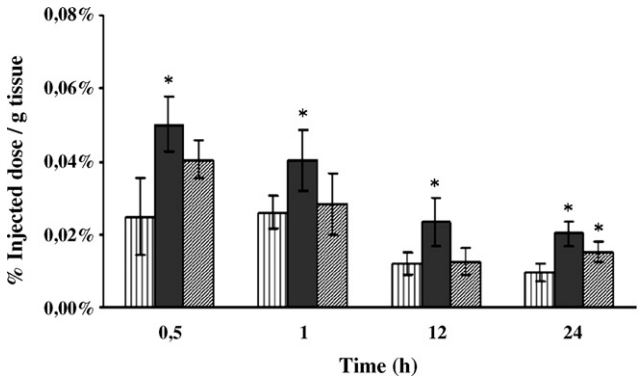

Fig. 5. Brain concentration (% injected dose/g tissue) of functionalized nanocapsules (vertically dashed columns), OX26-immuno-nanocapsules (closed columns) and Fab′-immuno-nanocapsules (right-dashed columns) at different times. Values represent means ± SEM ($n = 5$). *Statistically significant differences to functionalized nanocapsules (Mann–Whitney), $p < 0.05$ (inspired from (Beduneau et al., 2007a)).

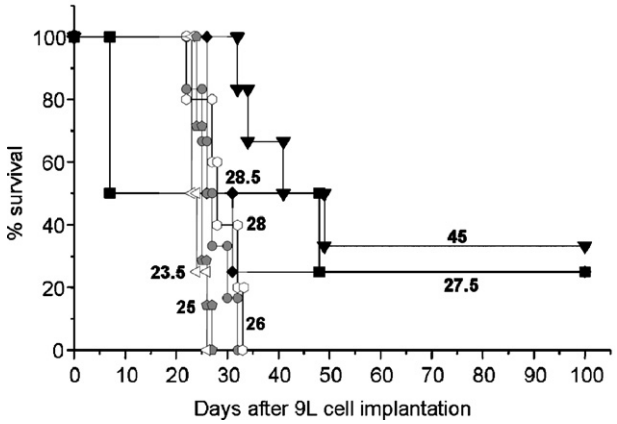

Fig. 6. Kaplan–Meier survival curve of different animal groups treated on Day 6 with 12 Gy ^{188}Re-SSS LNC ($n = 4$; black squares), 10 Gy ^{188}Re-SSS LNC ($n = 4$; black diamonds), 8 Gy ^{188}Re-SSS LNC ($n = 6$; black triangles), 3 Gy ^{188}Re-SSS LNC ($n = 5$; blank hexagons), 4 Gy ^{188}Re perrhenate ($n = 4$; blank triangles), blank LNC ($n = 7$; grey circles) or not treated ($n = 8$; grey pentagons). Numbers represent the median survival time of corresponding group (inspired from (Allard et al., 2008a)).

and rat brain cerebral endothelial cells (BCECs), as well as immuno-nanocapsule distribution in healthy rats after intravenous injection (Beduneau et al., 2008). Ligand density per immuno-nanocapsule has been adjusted between 30 and 40 OX26 MAb or Fab′ fragments. Experimental results from flow cytometry elicited the binding of immuno-nanocapsules to cells via TfR. Concerning biodistribution, as expected, Fab′-immuno-nanocapsules were retained longer in the bloodstream in comparison with OX26-immuno-nanocapsules which are quickly absorbed by the liver. On the contrary, brain accumulation of OX26-immuno-nanocapsules was more efficient than Fab′-immuno-nanocapsules (Fig. 5). However, both types of immuno-nanocapsules provided significant accumulation in the brain 24 h after administration when compared to non-targeted LNCs (2- and 1.5-fold higher respectively) (Beduneau et al., 2008).

Such immuno-nanocapsules represent promising nanocarriers for the active targeting of drug delivery to brain tumors. Further studies on brain tumor-bearing animal models, as well as drug encapsulation into immuno-nanocapsules, might be required to elucidate their targeting possibilities and their potential application in cancer treatment.

3.5. Local treatment

As mentioned above, the delivery of drugs across the BBB is very limited, leading to the failure of major conventional systemic chemotherapies in brain cancer. This is particularly the case of glioblastoma multiforme, this being the most prevalent of malignant glioma in adults, resulting in highly unfavorable survival prognoses (Ohgaki and Kleihues, 2005). Direct intracranial drug delivery by stereotaxic injection would eliminate the need for a chemotherapeutic agent to bypass the BBB. Among the different injection strategies, convection-enhanced delivery (CED) (Vogelbaum, 2005), using an external pressure gradient inducing fluid convection in the brain via a surgically implanted catheter, allows greater volume distribution to be achieved in comparison to diffusion alone (Bobo et al., 1994). Therefore, a combination of nanotechnology with the CED technique appears promising as an approach for direct drug delivery for the treatment of brain tumors (Sawyer et al., 2006; Allard et al., 2009).

In this way, local treatment with the ^{188}Re-SSS complex (^{188}Re$(S_3CPh)_2(S_2CPh)$)-loaded LNCs by means of CED on a 9L intracranial xenograft model of a rat brain tumor has been investigated (Allard et al., 2008a). The different doses of ^{188}Re (12, 10, 8 and 3 Gy) were evaluated based on animal survival time. Interestingly, the 8 Gy ^{188}Re-SSS LNC-treated group showed a significant improvement in median survival time as compared to a control group and a blank LNC-treated group (Fig. 6). The increase in the median survival time was about 80% compared to the control group, and 33% of the animals were long-term survivors (over 100 days). The dose of 8 Gy proved to be effective, between toxic (10–12 Gy) and ineffective (3–4 Gy) doses. In addition, this formulation of LNCs was eliminated more slowly than the classical solution of ^{188}Re (perrhenate $^{188}ReO_4^-$) which is recovered very quickly in the urine. LNCs ensured a prolonged therapeutic effect and can be considered as a promising radio-pharmaceutical carrier for internal radiotherapy of brain tumors.

The same method was recently used for the local delivery of LNCs loaded with ferrocenyl diphenol molecules called 'ferrociphenol' (Fc-diOH) followed by external beam irradiation on a rat brain tumor model (Allard et al., submitted for publication). The results showed a synergistic antiproliferative effect between Fc-diOH–LNCs and *in vitro* radiotherapy on 9L glioma cells as well as in an intracerebral *in vivo* 9L glioma model. Previously, these Fc-diOH–LNCs were shown to be cytotoxic on 9L glioma cells ($IC_{50} = 0.6\ \mu M$) and harmless on healthy brain cells up to a concentration range of 10 μM (Allard et al., 2008b). Moreover, in the same study, an antitumor effect was also obtained after a single intratumoral injection at Day 6 after subcutaneous 9L injection on Fischer F344 rats where Fc-diOH–LNCs treatment dramatically reduced both tumor mass and tumor volume.

3.6. Oral administration

Oral drug administration remains the preferred route of administration to ensure patient satisfaction and compliance because it is easy to handle and does not require medical assistance or equipment, and thereby does not necessitate hospitalization. However, it is evident that not all drugs can be administered orally due to the influence of various factors on their pharmacokinetic profile, such as physico-chemical properties, pharmaceutical factors, and physiological factors of the gastrointestinal system (Undevia et al., 2005). The presence of P-glycoprotein (P-gp) on the enterocyte surface limits oral bioavailability (Oostendorp et al., 2009).

As mentioned previously, paclitaxel is pumped out by P-gp which also limits the oral uptake of paclitaxel and mediates the direct excretion of the drug from the systemic circulation into the intestinal lumen (Sparreboom et al., 1997). Therefore, the strategy to enhance the bioavailability of paclitaxel by oral administration is very interesting and requires novel formulations. This issue can

also be addressed by the entrapment of drug molecules in the LNCs (Peltier et al., 2006). Indeed, by loading paclitaxel into the oily core of LNCs, its mean plasmatic concentration is 3 times higher compared to the conventional formulation (Taxol®) and is 1.5 times higher than the co-administration of Taxol® and verapamil, which is known as a major P-gp inhibitor.

Thus, such drug/carrier particulate systems provide an attractive and exciting drug delivery approach for highly potent drug substances that are usually unsuitable for oral use. Further studies will be carried out to elucidate the benefits of this approach compared to conventional drug formulations, their compatibility for large-scale industrial production, and the stringency of registration requirements (Wawrezinieck et al., 2008).

4. Conclusion

Lipid nanocapsules provide a new tool which contributes to nanomedicine development. Using FDA-approved constituents, LNCs are prepared by a solvent-free process to obtain particles of less than 100 nm with monodispersity. These LNCs provide considerable drug encapsulation capacity and also exhibit sustained-release functions at the site of action. Moreover, thanks to the polyoxyethylene-type nonionic surfactant surface, LNCs display a P-gp inhibitory effect harmonized with a stealth effect versus the complement system as well as MPS uptake; they also can be grafted with ligands for the purpose of actively targeting drug delivery.

The interesting possibilities cited in this article for the use of LNCs, allow their use in many therapeutic applications, not only for drug delivery, cancer diagnosis and therapy, but also for gene and cell therapy. Consequently, a generation of LNCs loaded with nucleic acid was recently investigated and patented (Table 1). This opened up an opportunity for the development of gene therapy via a non-viral vector, instead of viral vectors which present many limitations concerning safety, immunogenicity, low transgene size and high costs (Morille et al., 2008). In fact, hydrophilic DNA molecules can be encapsulated into the oily core of LNCs leading to the formation of neutral, 110-nm DNA nanocapsules (Vonarbourg et al., 2009). With the goal of increasing systemic delivery by making DNA LNCs stealthy, DNA LNCs were coated with amphiphilic and flexible PEG polymers [PEG lipid derivative (DSPE-mPEG 2000) or F108 poloxamer] (Morille et al., in 2009). Furthermore, to overcome the internalization difficulties encountered with PEG shields, active targeting using galactose has also been designed for the purpose of efficient hepatocyte targeting (Morille et al., in 2009).

Therefore, LNCs are also adequate systems to encapsulate lipophilic complexes of hydrophilic molecules. Nevertheless, an alternative could be the encapsulation of free hydrophilic molecules inside an aqueous core. New hydrophilic drug delivery systems have been developed in this way (see Table 1).

Acknowledgment

The authors would like to thank Archibald Paillard, Emilie Allard, Arnaud Beduneau, and Beatrice Heurtault for their help in drawing the figures.

References

Abismail, B., Canselier, J.P., Wilhelm, A.M., Delmas, H., Gourdon, C., 1999. Emulsification by ultrasound: drop size distribution and stability. Ultrason. Sonochem. 6, 75–83.

Allard, E., Hindre, F., Passirani, C., Lemaire, L., Lepareur, N., Noiret, N., Menei, P., Benoit, J.P., 2008a. ^{188}Re-loaded lipid nanocapsules as a promising radiopharmaceutical carrier for internal radiotherapy of malignant gliomas. Eur. J. Nucl. Med. Mol. Imaging 35, 1838–1846.

Allard, E., Passirani, C., Benoit, J.P., 2009. Convection-enhanced delivery of nanocarriers for the treatment of brain tumors. Biomaterials 30, 2302–2318.

Allard, E., Passirani, C., Garcion, E., Pigeon, P., Vessieres, A., Jaouen, G., Benoit, J.P., 2008b. Lipid nanocapsules loaded with an organometallic tamoxifen derivative as a novel drug-carrier system for experimental malignant gliomas. J. Control. Release 130, 146–153.

Allard, E., Passirani, C., Jarnet, D., Petit, S., Vessieres, A., Jaouen, G., Benoit, J.P., submitted for publication. Local delivery of ferrocifenol lipid nanocapsules followed by external radiotherapy as a synergistic treatment against intracranial 9L glioma xenograft. J. Pharmacol. Exp. Ther.

Anton, N., Benoit, J.P., Saulnier, P., 2008. Design and production of nanoparticles formulated from nano-emulsion templates—a review. J. Control. Release 128, 185–199.

Anton, N., Gayet, P., Benoit, J.P., Saulnier, P., 2007a. Nano-emulsions and nanocapsules by the PIT method: an investigation on the role of the temperature cycling on the emulsion phase inversion. Int. J. Pharm. 344, 44–52.

Anton, N., Saulnier, P., Beduneau, A., Benoit, J.P., 2007b. Salting-out effect induced by temperature cycling on a water/nonionic surfactant/oil system. J. Phys. Chem. B 111, 3651–3657.

Ballot, S., Noiret, N., Hindre, F., Denizot, B., Garin, E., Rajerison, H., Benoit, J.P., 2006. ^{99m}Tc/^{188}Re-labelled lipid nanocapsules as promising radiotracers for imaging and therapy: formulation and biodistribution. Eur. J. Nucl. Med. Mol. Imaging 33, 602–607.

Beduneau, A., Hindre, F., Clavreul, A., Leroux, J.C., Saulnier, P., Benoit, J.P., 2008. Brain targeting using novel lipid nanovectors. J. Control. Release 126, 44–49.

Beduneau, A., Saulnier, P., Anton, N., Hindre, F., Passirani, C., Rajerison, H., Noiret, N., Benoit, J.P., 2006. Pegylated nanocapsules produced by an organic solvent-free method: evaluation of their stealth properties. Pharm. Res. 23, 2190–2199.

Beduneau, A., Saulnier, P., Benoit, J.P., 2007a. Active targeting of brain tumors using nanocarriers. Biomaterials 28, 4947–4967.

Beduneau, A., Saulnier, P., Hindre, F., Clavreul, A., Leroux, J.C., Benoit, J.P., 2007b. Design of targeted lipid nanocapsules by conjugation of whole antibodies and antibody Fab′ fragments. Biomaterials 28, 4978–4990.

Bobo, R.H., Laske, D.W., Akbasak, A., Morrison, P.F., Dedrick, R.L., Oldfield, E.H., 1994. Convection-enhanced delivery of macromolecules in the brain. Proc. Natl. Acad. Sci. U.S.A. 91, 2076–2080.

Bouchemal, K., Briancon, S., Perrier, E., Fessi, H., 2004. Nano-emulsion formulation using spontaneous emulsification: solvent, oil and surfactant optimisation. Int. J. Pharm. 280, 241–251.

Brigger, I., Dubernet, C., Couvreur, P., 2002. Nanoparticles in cancer therapy and diagnosis. Adv. Drug Deliv. Rev. 54, 631–651.

Caruthers, S.D., Wickline, S.A., Lanza, G.M., 2007. Nanotechnological applications in medicine. Curr. Opin. Biotechnol. 18, 26–30.

Coon, J.S., Knudson, W., Clodfelter, K., Lu, B., Weinstein, R.S., 1991. Solutol HS, 15, nontoxic polyoxyethylene esters of 12-hydroxystearic acid, reverses multidrug resistance. Cancer Res. 51, 897–902.

Dulieu, C., Bazile, D., 2005. Influence of lipid nanocapsules composition on their aptness to freeze-drying. Pharm. Res. 22, 285–292.

Fenart, L., Casanova, A., Dehouck, B., Duhem, C., Slupek, S., Cecchelli, R., Betbeder, D., 1999. Evaluation of effect of charge and lipid coating on ability of 60-nm nanoparticles to cross an in vitro model of the blood–brain barrier. J. Pharmacol. Exp. Ther. 291, 1017–1022.

Floury, J., Desrumaux, A., Axelos, M.A.V., Legrand, J., 2003. Effect of high pressure homogenisation on methylcellulose as food emulsifier. J. Food Eng. 58, 227–238.

Friberg, S., Lapczynska, I., Gillberg, G., 1976. Microemulsions containing nonionic surfactants—the importance of the pit value. J. Colloids Interface Sci. 56, 19–32.

Friden, P.M., Walus, L.R., Musso, G.F., Taylor, M.A., Malfroy, B., Starzyk, R.M., 1991. Anti-transferrin receptor antibody and antibody–drug conjugates cross the blood–brain barrier. Proc. Natl. Acad. Sci. U.S.A. 88, 4771–4775.

Garcion, E., Lamprecht, A., Heurtault, B., Paillard, A., Aubert-Pouessel, A., Denizot, B., Menei, P., Benoit, J.P., 2006. A new generation of anticancer, drug-loaded, colloidal vectors reverses multidrug resistance in glioma and reduces tumor progression in rats. Mol. Cancer Ther. 5, 1710–1722.

Ghofrani, H.A., Osterloh, I.H., Grimminger, F., 2006. Sildenafil: from angina to erectile dysfunction to pulmonary hypertension and beyond. Nat. Rev. Drug Discov. 5, 689–702.

Haley, B., Frenkel, E., 2008. Nanoparticles for drug delivery in cancer treatment. Urol. Oncol. 26, 57–64.

Hall, W.A., 1991. Transferrin receptor on glioblastoma multiforme. J. Neurosurg. 74, 313–314.

Heurtault, B., Saulnier, P., Pech, B., Benoit, J.P., Proust, J.E., 2003a. Interfacial stability of lipid nanocapsules. Colloids Surf. B: Biointerfaces 30, 225–235.

Heurtault, B., Saulnier, P., Pech, B., Proust, J.E., Benoit, J.P., 2002a. A novel phase inversion-based process for the preparation of lipid nanocarriers. Pharm. Res. 19, 875–880.

Heurtault, B., Saulnier, P., Pech, B., Proust, J.E., Benoit, J.P., 2002b. Properties of polyethylene glycol 660 12-hydroxy stearate at a triglyceride/water interface. Int. J. Pharm. 242, 167–170.

Heurtault, B., Saulnier, P., Pech, B., Proust, J.E., Benoit, J.P., 2003b. Physico-chemical stability of colloidal lipid particles. Biomaterials 24, 4283–4300.

Heurtault, B., Saulnier, P., Pech, B., Proust, J.E., Richard, J., Benoit, J.P., 2000. Lipidic nanocapsules: preparation process and use as Drug Delivery Systems. Patent No. WO02688000.

Heurtault, B., Saulnier, P., Pech, B., Venier-Julienne, M.C., Proust, J.E., Phan-Tan-Luu, R., Benoit, J.P., 2003c. The influence of lipid nanocapsule composition on their size distribution. Eur. J. Pharm. Sci. 18, 55–61.

Hoarau, D., Delmas, P., David, S., Roux, E., Leroux, J.C., 2004. Novel long-circulating lipid nanocapsules. Pharm. Res. 21, 1783–1789.
Jallouli, Y., Paillard, A., Chang, J., Sevin, E., Betbeder, D., 2007. Influence of surface charge and inner composition of porous nanoparticles to cross blood–brain barrier in vitro. Int. J. Pharm. 344, 103–109.
Jefferies, W.A., Brandon, M.R., Hunt, S.V., Williams, A.F., Gatter, K.C., Mason, D.Y., 1984. Transferrin receptor on endothelium of brain capillaries. Nature 312, 162–163.
Jestin, E., Mougin-Degraef, M., Faivre-Chauvet, A., Remaud-Le Saec, P., Hindre, F., Benoit, J.P., Chatal, J.F., Barbet, J., Gestin, J.F., 2007. Radiolabeling and targeting of lipidic nanocapsules for applications in radioimmunotherapy. Q. J. Nucl. Med. Mol. Imaging 51, 51–60.
Khalid, M.N., Simard, P., Hoarau, D., Dragomir, A., Leroux, J.C., 2006. Long circulating poly(ethylene glycol)-decorated lipid nanocapsules deliver docetaxel to solid tumors. Pharm. Res. 23, 752–758.
Lacoeuille, F., Hindre, F., Moal, F., Roux, J., Passirani, C., Couturier, O., Cales, P., Le Jeune, J.J., Lamprecht, A., Benoit, J.P., 2007. In vivo evaluation of lipid nanocapsules as a promising colloidal carrier for paclitaxel. Int. J. Pharm. 344, 143–149.
Lamprecht, A., Benoit, J.P., 2006. Etoposide nanocarriers suppress glioma cell growth by intracellular drug delivery and simultaneous P-glycoprotein inhibition. J. Control. Release 112, 208–213.
Lamprecht, A., Bouligand, Y., Benoit, J.P., 2002. New lipid nanocapsules exhibit sustained release properties for amiodarone. J. Control. Release 84, 59–68.
Lamprecht, A., Saumet, J.L., Roux, J., Benoit, J.P., 2004. Lipid nanocarriers as drug delivery system for ibuprofen in pain treatment. Int. J. Pharm. 278, 407–414.
Letchford, K., Burt, H., 2007. A review of the formation and classification of amphiphilic block copolymer nanoparticulate structures: micelles, nanospheres, nanocapsules and polymersomes. Eur. J. Pharm. Biopharm. 65, 259–269.
Maeda, H., Wu, J., Sawa, T., Matsumura, Y., Hori, K., 2000. Tumor vascular permeability and the EPR effect in macromolecular therapeutics: a review. J. Control. Release 65, 271–284.
Malzert-Freon, A., Vrignaud, S., Saulnier, P., Lisowski, V., Benoit, J.P., Rault, S., 2006. Formulation of sustained release nanoparticles loaded with a tripentone, a new anticancer agent. Int. J. Pharm. 320, 157–164.
Manconi, M., Aparicio, J., Vila, A.O., Pendas, J., Figueruelo, J., Molina, F., 2003. Viscoelastic properties of concentrated dispersions in water of soy lecithin. Colloids Surf. A: Physicochem. Eng. Aspects 222, 141–145.
Maruyama, K., Takahashi, N., Tagawa, T., Nagaike, K., Iwatsuru, M., 1997. Immunoliposomes bearing polyethyleneglycol-coupled Fab′ fragment show prolonged circulation time and high extravasation into targeted solid tumors in vivo. FEBS Lett. 413, 177–180.
Miller, D.J., Henning, T., Grunbein, W., 2001. Phase inversion of W/O emulsions by adding hydrophilic surfactant—a technique for making cosmetics products. Colloids Surf. A: Physicochem. Eng. Aspects 183–185, 681–688.
Minkov, I., Ivanova, T., Panaiotov, I., Proust, J., Saulnier, P., 2005. Reorganization of lipid nanocapsules at air-water interface. Part 2. Properties of the formed surface film. Colloids Surf. B: Biointerfaces 44, 197–203.
Modok, S., Mellor, H.R., Callaghan, R., 2006. Modulation of multidrug resistance efflux pump activity to overcome chemoresistance in cancer. Curr. Opin. Pharmacol. 6, 350–354.
Moghimi, S.M., Hunter, A.C., Murray, J.C., 2001. Long-circulating and target-specific nanoparticles: theory to practice. Pharmacol. Rev. 53, 283–318.
Moghimi, S.M., Szebeni, J., 2003. Stealth liposomes and long circulating nanoparticles: critical issues in pharmacokinetics, opsonization and protein-binding properties. Prog. Lipid Res. 42, 463–478.
Morales, D., Gutierrez, J.M., Garcia-Celma, M.J., Solans, Y.C., 2003. A study of the relation between bicontinuous microemulsions and oil/water nano-emulsion formation. Langmuir 19, 7196–7200.
Morille, M., Passirani, C., Letrou-Bonneval, E., Benoit, J.P., Pitard, B., 2009. Galactosylated DNA lipid nanocapsules for an efficient hepatocyte targeting. Int. J. Pharm. 379, 293–300.
Morille, M., Passirani, C., Vonarbourg, A., Clavreul, A., Benoit, J.P., 2008. Progress in developing cationic vectors for non-viral systemic gene therapy against cancer. Biomaterials 29, 3477–3496.
Mosqueira, V.C., Legrand, P., Gulik, A., Bourdon, O., Gref, R., Labarre, D., Barratt, G., 2001a. Relationship between complement activation, cellular uptake and surface physicochemical aspects of novel PEG-modified nanocapsules. Biomaterials 22, 2967–2979.
Mosqueira, V.C., Legrand, P., Morgat, J.L., Vert, M., Mysiakine, E., Gref, R., Devissaguet, J.P., Barratt, G., 2001b. Biodistribution of long-circulating PEG-grafted nanocapsules in mice: effects of PEG chain length and density. Pharm. Res. 18, 1411–1419.
Naccarelli, G.V., Rinkenberger, R.L., Dougherty, A.H., Giebel, R.A., 1985. Amiodarone: pharmacology and antiarrhythmic and adverse effects. Pharmacotherapy 5, 298–313.
Ohgaki, H., Kleihues, P., 2005. Epidemiology and etiology of gliomas. Acta Neuropathol. 109, 93–108.
Oostendorp, R.L., Beijnen, J.H., Schellens, J.H., 2009. The biological and clinical role of drug transporters at the intestinal barrier. Cancer Treat. Rev. 35, 137–147.
Osterloh, I.H., Riley, A., 2002. Clinical update on sildenafil citrate. Br. J. Clin. Pharmacol. 53, 219–223.
Pardridge, W.M., 2005. The blood–brain barrier: bottleneck in brain drug development. NeuroRx 2, 3–14.
Pardridge, W.M., Buciak, J.L., Friden, P.M., 1991. Selective transport of an anti-transferrin receptor antibody through the blood-brain barrier in vivo. J. Pharmacol. Exp. Ther. 259, 66–70.
Passirani, C., Benoit, J.P., 2005. Complement activation by injectable colloidal drug carriers. In: Biomaterials for Delivery and Targeting of Proteins and Nucleic Acids. CRC Press, Inc., Boca Raton, pp. 187–230.
Peltier, S., Oger, J.M., Lagarce, F., Couet, W., Benoit, J.P., 2006. Enhanced oral paclitaxel bioavailability after administration of paclitaxel-loaded lipid nanocapsules. Pharm. Res. 23, 1243–1250.
Pereira de Oliveira, M., Garcion, E., Venisse, N., Benoit, J.P., Couet, W., Olivier, J.C., 2005. Tissue distribution of indinavir administered as solid lipid nanocapsule formulation in mdr1a (+/+) and mdr1a (−/−) CF-1 mice. Pharm. Res. 22, 1898–1905.
Saulnier, P., Anton, N., Heurtault, B., Benoit, J.-P., 2008. Liquid crystals and emulsions in the formulation of drug carriers. Comptes Rendus Chimie 11, 221–228.
Sawyer, A.J., Piepmeier, J.M., Saltzman, W.M., 2006. New methods for direct delivery of chemotherapy for treating brain tumors. Yale J. Biol. Med. 79, 141–152.
Shah, J.C., Chen, J.R., Chow, D., 1989. Preformulation study of etoposide: identification of physicochemical characteristics responsible for the low and erratic oral bioavailability of etoposide. Pharm. Res. 6, 408–412.
Shinoda, K., Saito, H., 1969. The stability of O/W type emulsions as functions of temperature and the HLB of emulsifiers: the emulsification by PIT-method. J. Colloids Interface Sci. 30, 258–263.
Solans, C., Izquierdo, P., Nolla, J., Azemar, N., Garcia-Celma, M.J., 2005. Nano-emulsions. Curr. Opin. Colloids Interface Sci. 10, 102–110.
Sparreboom, A., Nooter, K., 2000. Does P-glycoprotein play a role in anticancer drug pharmacokinetics? Drug Resist. Updat. 3, 357–363.
Sparreboom, A., van Asperen, J., Mayer, U., Schinkel, A.H., Smit, J.W., Meijer, D.K., Borst, P., Nooijen, W.J., Beijnen, J.H., van Tellingen, O., 1997. Limited oral bioavailability and active epithelial excretion of paclitaxel (Taxol) caused by P-glycoprotein in the intestine. Proc. Natl. Acad. Sci. U.S.A. 94, 2031–2035.
Steiniger, S.C., Kreuter, J., Khalansky, A.S., Skidan, I.N., Bobruskin, A.I., Smirnova, Z.S., Severin, S.E., Uhl, R., Kock, M., Geiger, K.D., Gelperina, S.E., 2004. Chemotherapy of glioblastoma in rats using doxorubicin-loaded nanoparticles. Int. J. Cancer. 109, 759–767.
Tadros, T., Izquierdo, P., Esquena, J., Solans, C., 2004. Formation and stability of nano-emulsions. Adv. Colloids Interface Sci. 108–109, 303–318.
Undevia, S.D., Gomez-Abuin, G., Ratain, M.J., 2005. Pharmacokinetic variability of anticancer agents. Nat. Rev. Cancer 5, 447–458.
Vogelbaum, M.A., 2005. Convection enhanced delivery for the treatment of malignant gliomas: symposium review. J. Neuro-Oncol. 73, 57–69.
Vonarbourg, A., Saulnier, P., Passirani, C., Benoit, J.P., 2005. Electrokinetic properties of noncharged lipid nanocapsules: influence of the dipolar distribution at the interface. Electrophoresis 26, 2066–2075.
Vonarbourg, A., Passirani, C., Saulnier, P., Benoit, J.P., 2006a. Parameters influencing the stealthiness of colloidal drug delivery systems. Biomaterials 27, 4356–4373.
Vonarbourg, A., Passirani, C., Saulnier, P., Simard, P., Leroux, J.C., Benoit, J.P., 2006b. Evaluation of pegylated lipid nanocapsules versus complement system activation and macrophage uptake. J. Biomed. Mater. Res. A 78, 620–628.
Vonarbourg, A., Passirani, C., Desigaux, L., Allard, E., Saulnier, P., Lambert, O., Benoit, J.P., Pitard, B., 2009. The encapsulation of DNA molecules within biomimetic lipid nanocapsules. Biomaterials. 30, 3197–3204.
Wawrezinieck, A., Pean, J.-M., Wuthrich, P., Benoit, J.-P., 2008. Oral bioavailability and drug/carrier particulate systems. Medicine/Sciences 24, 659–664.
Wehrlé, P., 2007. Chapitre 3: liposomes. In: Pharmacie galénique: formulation et technologie pharmaceutique. Maloine, pp. 241–250.
Winsor, P., 1954. Solvent Properties of Amphiphilic Compounds. Butterworth Londres.
Working, P.K., Dayan, A.D., 1996. Pharmacological-toxicological Expert Report. Caelyx®. (Stealth® liposomal doxorubicin HCl). Human Exp. Toxicol. 15, 751–785.