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Nanoparticles in cancer therapy and diagnosis

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Abstract

Numerous investigations have shown that both tissue and cell distribution profiles of anticancer drugs can be controlled by their entrapment in submicronic colloidal systems (nanoparticles). The rationale behind this approach is to increase antitumor efficacy, while reducing systemic side-effects. This review provides an update of tumor targeting with conventional or long-circulating nanoparticles. The in vivo fate of these systems, after intravascular or tumoral administration, is discussed, as well as the mechanism involved in tumor regression. Nanoparticles are also of benefit for the selective delivery of oligonucleotides to tumor cells. Moreover, certain types of nanoparticles showed some interesting capacity to reverse MDR resistance, which is a major problem in chemotherapy. The first experiments, aiming to decorate nanoparticles with molecular ligand for 'active' targeting of cancerous cells, are also discussed here. The last part of this review focus on the application of nanoparticles in imaging for cancer diagnosis.

Keywords: Nanoparticles; Nanospheres; Nanocapsules; Conventional or long-circulating carriers; Passive or active tumor targeting; EPR effect; Multidrug resistance (MDR); Oligonucleotide delivery; Tumor imaging

Contents

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1. Introduction

Neoplastic tissues may be divided into three subcompartments: vascular, interstitial and cellular [1].

The vascularization of tumors is heterogeneous, showing regions of necrosis or hemorrhages as well as regions which are densely vascularized in order to sustain an adequate supply of nutrients and oxygen for rapid tumor growth (angiogenesis) [2]. Tumor blood vessels present several abnormalities in comparison with normal physiological vessels, often including a relatively high proportion of proliferating endothelial cells, an increased tortuosity, a deficiency in pericytes and an aberrant basement membrane formation [3,4]. The resulting enhanced permeability of tumor vasculature is thought to be regulated by various mediators, such as vascular endothelium growth factor (VEGF), bradykinin, nitric oxide, prostaglandins and matrix metalloproteinases [5]. Macromolecular transport pathways across tumor vessels have been shown to occur via open gaps (interendothelial junctions and transendothelial channels), vesicular vacuolar organelles (VVO) and fenestrations [6]. It remains, however, controversial as to which pathways are predominantly responsible for tumor hyperpermeability and macromolecular transvascular transport [6]. Regardless of the transport mechanism, the pore cutoff size of several tumor models has been reported ranging between 380 and 780 nm [6,7]. In vivo fluorescence microscopy has even permitted direct measurement of the extravasation of sterically stabilized liposomes into solid tumor tissue (neuroblastome C-1300), suggesting that the cutoff size of the pores lies around 400 nm [8].

The tumor interstitial compartment is predominantly composed of a collagen and elastic fiber network [1]. Interdispersed within this cross-linked structure are the interstitial fluid and macromolecular constituents (hyaluronate and proteoglycans), which form a hydrophilic gel [1]. The interstitium, unlike most normal tissues, is also characterized by a high interstitial pressure leading to an outward convective interstitial fluid flow, as well as the absence of an anatomically well-defined functioning lymphatic network [1,2]. Hence, the transport of an anticancer drug in the interstitium will be governed by physiological (i.e. pressure) and physicochemical (i.e. composition, structure, charge) properties of the interstitium and by the physicochemical properties of the molecule (size, configuration, charge, hydrophobicity) itself [1].

Thus, to deliver therapeutic agents to tumor cells in vivo, one must overcome the following problems: (i) drug resistance at the tumor level due to physiological barriers (non cellular based mechanisms), (ii) drug resistance at the cellular level (cellular mechanisms), and (iii) distribution, biotransformation and clearance of anticancer drugs in the body.

In chemotherapy, clinical drug resistance may be defined either as a lack of tumor size reduction or as the occurrence of clinical relapse after an initial positive response to anti-tumor treatment [9].

First, non-cellular drug resistance mechanisms could be due to poorly vascularized tumor regions which can effectively reduce drug access to the tumor and thus protect cancerous cells from cytotoxicity. The acidic environment in tumors can also confer a resistance mechanism against basic drugs. These compounds would be ionized, preventing their diffusion across cellular membrane. High interstitial pressure and low microvascular pressure may also retard or impede extravasation of molecules [10].

Then, the resistance of tumors to therapeutic intervention may be due to cellular mechanisms, which are categorized in term of alterations in the biochemistry of malignant cells. They comprise altered activity of specific enzyme systems (for example topoisomerase activity), altered apoptosis regulation, or transport based mechanisms, like P-glycoprotein efflux system, responsible for the multidrug resistance (MDR), or the multi-drug resistance associated protein (MRP) [9,10].

Finally, anticancer drugs generally feature large

volumes of distribution. As cancer fighting drugs are toxic to both tumor and normal cells, the efficacy of chemotherapy is often limited by important side-effects.

A strategy could be to associate antitumor drugs with colloidal nanoparticles, with the aim to overcome non-cellular and cellular based mechanisms of resistance and to increase selectivity of drugs towards cancer cells while reducing their toxicity towards normal tissues.

Nanoparticles may be defined as being submicronic (< 1 μm) colloidal systems generally, but not necessarily, made of polymers (biodegradable or not). According to the process used for the preparation of the nanoparticles, nanospheres or nanocapsules can be obtained. Unlike nanospheres (matrix systems in which the drug is dispersed throughout the particles), nanocapsules are vesicular systems in which the drug is confined to an aqueous or oily cavity surrounded by a single polymeric membrane. Nanocapsules may, thus, be considered as a 'reservoir' system (Fig. 1) [11]

If designed appropriately, nanoparticles may act as a drug vehicle able to target tumor tissues or cells, to a certain extent, while protecting the drug from premature inactivation during its transport.

Indeed, at the tumor level, the accumulation mechanism of intravenously injected nanoparticles relies on a passive diffusion or convection across the leaky, hyperpermeable tumor vasculature [12]. The uptake can also result from a specific recognition in case of ligand decorated nanoparticles ('active targeting') [13].

Hence, as the clearance via lymphatics is generally seriously compromised in neoplastic tissues, there will be an additional retention of the colloidal particles (or macromolecules with a molecular weight above 50 kDa) in the tumor interstitium. This particular concept denominated 'enhanced permeability and retention effect' (EPR) results in an important intratumoral drug accumulation which is even higher than this observed in plasma and other tissues [5,14,15].

Furthermore, a controlled release of the drug content inside of the tumoral interstitium may be

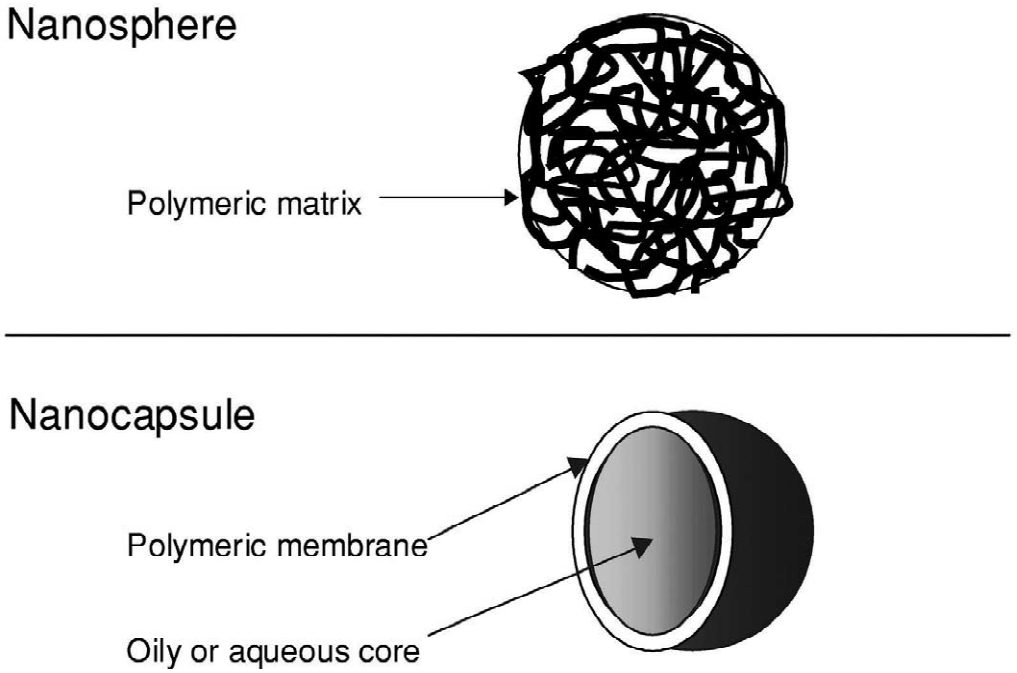


Fig. 1. Nanoparticles are: nanosphere (matrix systems) (top) or nanocapsule (reservoir system) (bottom).

achieved by controlling the nanoparticulate structure: polymers used and the way by which the drug is associated with the carrier (adsorption or encapsulation).

This paper will first review how nanoparticles loaded with anticancer drug successfully increase drug concentration in cancer tissues, enhancing antitumor efficacy.

Moreover, nanoparticles may also act at the cellular level. They can be endocytosed/phagocytosed by cells, with a resulting cell internalization of the encapsulated drug. Certain types of nanoparticles were also found to be able to overcome MDR resistance, which is due to the presence of the P-glycoprotein efflux system localized at the cancerous cell membrane. These points will be discussed in the second part of the paper.

Finally, apart from therapeutic goals, nanoparticles have also proved to be useful for diagnostic purpose. This will be presented in the last part of this review.

2. Nanoparticles for tumor tissues targeting and delivery

2.1. Systemic administration

2.1.1. Conventional nanoparticles

The association of a cytostatic drug to conventional carriers leads to modifications of the drug biodistribution profile, as it is mainly delivered to the mononuclear phagocytes system (MPS) (liver, spleen, lungs and bone marrow). Indeed, once in the bloodstream, surface non-modified nanoparticles (conventional nanoparticles) are rapidly opsonized and massively cleared by the fixed macrophages of the MPS organs [16].

This was demonstrated in mice treated with doxorubicin incorporated into poly(isohexylcyanoacrylate) (PIHCA) nanospheres, where higher concentrations of doxorubicin were found in the liver, spleen and lungs, as compared to the counterpart mice treated with free doxorubicin [17]. At the same time, the concentration of doxorubicin in the heart and kidneys of mice were lower than when free doxorubicin was used.

In much the same fashion, when actinomycin D was adsorbed on poly(methylcyanoacrylate) (PMCA) nanospheres, it concentrated mainly in the lungs of the rats [18]. However, when this compound was incorporated into the more slowly biodegradable poly(ethylcyanoacrylate) (PECA) nanospheres, the drug accumulated mainly in the small intestine of rats [19]. Finally, when vinblastine was incorporated into the same PECA nanospheres, the drug concentrated highly in the spleen of rats [19].

Thus, both the polymeric composition (type, hydrophobicity, biodegradation profile) of the nanoparticles and the associated drug (molecular weight, charge, localization in the nanospheres: adsorbed or incorporated) have a great influence on the drug distribution pattern in the reticuloendothelial organs [19]. However, the exact underlying mechanism was not fully understood, but it was observed that this effect was rapid (within 0.5 or 3 h) and compatible with endocytosis [19].

Such propensity of MPS macrophages for endocytosis/phagocytosis provides an opportunity to efficiently deliver therapeutic agents to these cells, using conventional nanoparticles. This biodistribution can be of benefit for the chemotherapeutic treatment of MPS localized tumors (for example, hepatocarcinoma or hepatic metastasis arising from digestive tract or gynaecological cancers, bronchopulmonary tumors (primitive tumors or metastasis) including ‘non small cells tumor’ and ‘small cells tumors’, myeloma and leukemia).

The increased antitumor efficacy when dealing with drugs associated to conventional nanoparticles was demonstrated on an hepatic metastases model in mice (M 5076 reticulum cell sarcoma) [20]. Doxorubicin–PIHCA nanoparticles had an improved antimetastatic efficacy, since their use resulted in a greater reduction of the number of metastases than when free doxorubicin was used [20]. Additionally, it appeared to increase the life span of the metastasis-bearing mice [20]. The underlying mechanism responsible for the increased therapeutic efficacy of the nanoparticle formulation was a transfer of doxorubicin from the healthy hepatic tissue, acting as a drug reservoir, to the malignant tissues [21]. Histological examination allowed to visualize a considerable accumulation of nanoparticles in the lysosomal vesicles of Kupffer cells, whereas nanoparticles could

not be clearly identified in tumoral cells [21]. Thus, Kupffer cells, after a massive uptake of nanoparticles by phagocytosis, were able to induce the release of doxorubicin, leading to a gradient of drug concentration, favorable for a prolonged diffusion of the free and still active drug towards the neighboring metastatic cells [21]. Finally, in vitro experiments permitted to rule out the mechanism by which doxorubicin itself or PIHCA nanoparticles could have potentiated the intrinsic tumoricidal effect of macrophages [22].

When conventional nanoparticles are used as carriers in chemotherapy, some cytotoxicity against the Kupffer cells or other targeted macrophages can be expected, as the class of drugs being used is able to induce apoptosis in these cells [13,23]. Treatments featuring frequent administrations (with intervals shorter than 2 weeks, period of restoration of Kupffer cells) could result in a deficiency of Kupffer cells, which in turn could lead to a decreased liver uptake, and a subsequent decreased therapeutic efficacy for hepatic tumors [13]. A risk for bacteriemia can also not to be excluded [13,23].

Moreover, conventional carriers also target the bone marrow (MPS organ), which is already an important but unfavorable site of action for most anticancer drugs. Hence, chemotherapy with such carriers may increase myelosuppressive effects. This was indeed observed with doxorubicin incorporated into poly(isobutylcyanoacrylate) (PIBCA) and PIHCA nanospheres, whose hematopoietic toxicity was generally more pronounced and long-lasting than that of free doxorubicin [24].

Acute renal toxicity was another murine-reported doxorubicin toxicity, which was amplified by the association of the drug to PIBCA nanospheres. This toxicity (proteinuria) on the kidneys was probably the result of a modified biodistribution of the associated drug, leading to a strong uptake by mesangial cells, which resulted in glomerular damage [25].

However, despite the amplification of these side-effects, it is likely that conventional nanoparticles have a better safety profile than free anticancer agents, when acting on normal tissues. For example, reduction of cardiac accumulation of drugs [26], as well as of genotoxicity of mitomycin C and farmorubicin [27] has been related. The former point is of great interest for chemotherapy with doxorubicin, as the limiting side effect of this compound is its cardiotoxity.

Clinical pharmacokinetics after a single intravenous administration of doxorubicin adsorbed onto polymethacrylate nanospheres has been investigated in hepatoma patients. This type of conventional carrier, although of limited use in vivo because not biodegradable, allowed to reduce both the volume of distribution and the elimination half-life of doxorubicin [28]. Again, these data are consistent with the uptake by MPS organs acting as a drug reservoir.

Another phase I clinical investigation has been carried out on 21 assessable patients with doxorubicin associated to biodegradable PIHCA nanospheres. Pharmacokinetic studies conducted in 3/21 patients revealed important interindividual variations for doxorubicin (encapsulated or not) plasma levels. Clinical toxicity of encapsulated doxorubicin consisted in dose-dependent myelosuppression of different grades in all patients (limiting toxicity), in pseudo-allergic reactions in 3/21 patients and in diffuse bone pain in 3/21 patients. However, neither cardiac toxicity nor hepatotoxicity were encountered among the 18 patients treated with the nanoparticle formulation. The former could be explained by the rather low cumulative dose administered (180 mg/m^2). It was important to check the later, as the treatment schedule was a repeated administration with 28 days interval between each cycles. Unfortunately, according to WHO criteria, there were only 2/21 stable diseases lasting 4–6 months. All the other patients had progressive disease after the first course of doxorubicin-loaded PIHCA nanospheres [29]. This could be due to tumor localization, as they were rarely located at MPS sites, resulting in subtherapeutic anticancer drug concentration exposure.

Consequently, the contribution of conventional nanoparticles to enhance anticancer drugs efficacy is limited to targeting tumors at the level of MPS organs. Addressing anticancer drug-loaded nanoparticles to other tumoral tissues is not feasible, due to their very short circulation time (the mean half-life of conventional nanoparticles is 3–5 min after intravenous administration). Besides, penetration of such a carrier system across the leaky tumoral

endothelium would be derisory, leading to subtherapeutic concentrations of the drug near the neoplastic cells.

2.1.2. Long-circulating nanoparticles

Since the usefulness of conventional nanoparticles is limited by their massive capture by the macrophages of the MPS after intravenous administration, other nanoparticulate devices must be considered to target tumors, which are not localized in the MPS area. Recently, a great deal of work has been devoted to developing so-called 'Stealth™' particles, which are 'invisible' to macrophages [30]. These Stealth™ nanoparticles have been shown to be characterized by a prolonged half-life in the blood compartment [31,32]. This allows them to selectively extravasate in pathological sites, like tumors or inflamed regions with a leaky vasculature (Fig. 2) [13]. As a result, such long-circulating nanoparticles are supposed to be able to directly target most tumors located outside the MPS regions [13].

The size of the colloidal carriers as well as their surface characteristics are the key for the biological fate of nanoparticles, since these parameters can prevent their uptake by MPS macrophages. A high curvature (resulting in a small size: <100 nm) and/or a hydrophilic surface (as opposed to the hydrophobic surface of conventional nanoparticles) are needed, in order to reduce opsonization reactions and subsequent clearance by macrophages [30].

Chemotherapy with small-sized nanoparticles was performed in tumor-bearing animals. Taxol incorporated into polyvinylpyrrolidone nanospheres with a diameter of 50–60 nm were assayed on a B16F10 murine melanoma transplanted subcutaneously in mice. Mice treated with repeated intravenous injections of taxol-loaded nanospheres showed a significant tumor regression and higher survival rates than mice treated with free taxol [33]. Similarly, the use in a murine tumor model (implanted subcutaneously J774A.1 macrophages) of a dextran–doxorubicin conjugate incorporated into small chitosan nanospheres (100 nm in diameter) was reported to outperform the free conjugate, especially in relation of life expectancy [34]. In both cases, a higher tumor uptake, thanks to the small size and the hydrophilicity of the carrier device, as well as a sustained release of the drug, were supposed to be the key factors in improving the efficacy of the chemotherapy [33,34].

A major breakthrough in the nanoparticles field consisted in using hydrophilic polymers, (poly(ethylene glycol) (PEG), poloxamines, poloxamers,

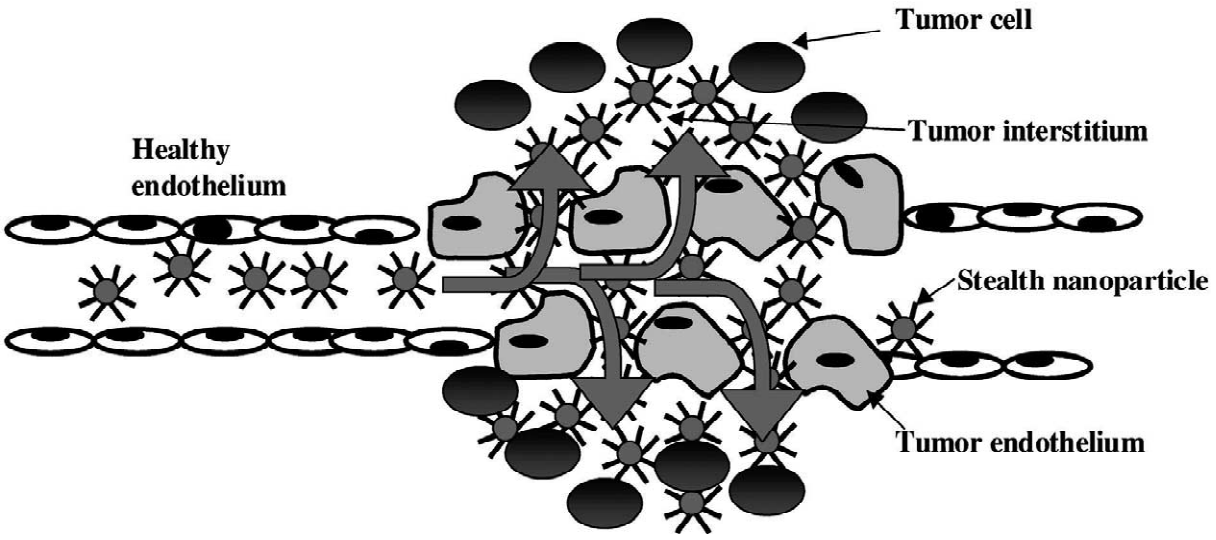


Fig. 2. Extravasation of long-circulating (Stealth™) nanoparticles in the tumor interstitium by passive diffusion or convection across the altered and hyperpermeable neoplastic endothelium.

polysaccharides) to efficiently coat conventional nanoparticle surface [30,35]. These coatings provide a dynamic 'cloud' of hydrophilic and neutral chains at the particle surface, which repel plasma proteins, as modeled by Jeon et al. [36,37]. Hydrophilic polymers can be introduced at the surface in two ways, either by adsorption of surfactants or by use of block or branched copolymers [30,31].

Coating conventional nanoparticles with surfactants, in order to obtain a long-circulating carrier, has been the first strategy used to direct tumor targeting in vivo.

For instance, chemotherapy was performed in B16 melanoma-bearing mice, with intravenous injection of mitoxantrone adsorbed onto PIBCA nanospheres, coated or not with poloxamine 1508 [38]. In both cases, the observed tumor concentrations of mitoxantrone were high. However, the influence of the hydrophilic coating of the nanoparticles on the biodistribution and pharmacokinetics were negligible and difficult to interpret, as the results showed important standard deviations. Moreover, the non-adsorbed drug (which accounted for 90% of the total drug) was not removed from the nanoparticles preparation and the hydrophilic coating was rapidly desorbed in vivo [38].

Another study was done using Polysorbate 80-coated PIBCA nanospheres [39]. Unfortunately, the coating again did not provide major changes in biodistribution and in pharmacokinetic parameters in rats, when compared to those of conventional PIBCA nanospheres. However, the coated nanospheres did transport a significant amount of incorporated doxorubicin to the brain of healthy rats, the highest drug levels (6 μg/g) being reached in this organ 2–4 h after intravenous administration. At that time, plasma concentration laid around 0.1 μg/g [39,40]. Consequently, it was suspected that an active transport from the blood to the brain took place, since cerebral accumulation occurred against a concentration gradient. A mechanism involving an endocytosis by brain endothelial cells was hypothesized, as the brain uptake was inhibited at 4 °C and after a pretreatment with cytochalasin B [39,40]. Finally, a therapeutic effect with this 'sterically stabilized' carrier system was noted in rats bearing intracranial glioblastoma (101/8 neoplastic cells) [41].

Very recently, other results were obtained with the non-biodegradable poly(methylmethacrylate) (PMMA) nanospheres coated or not with different surfactants (Polysorbate 80, Poloxamer 407 and Poloxamine 908). These systems were tested in mice, for their distribution in several tumor models, including a murine B16-melanoma (inoculated intramuscularly), a human breast cancer MaTu (engrafted subcutaneously) and an U-373 glioblastoma (implanted intracerebrally) [42]. The results showed a prolonged half life of the coated PMMA nanospheres in the circulation, especially with the Poloxamer 407 and the Poloxamine 908 coatings. Moreover an accumulation and retention of the coated PMMA nanospheres in the B-16 and MaTu tumors were observed; this accumulation depended on the particles surface hydrophilicity and the specific growth difference of the tumors [42]. Unfortunately, these experiments were performed without transcardiac perfusion with NaCl, which makes it difficult to separate the nanoparticles really accumulated in the tumor interstitium from those still present in the blood compartment.

Despite of these interesting results, the second strategy consisting in the covalent linkage of amphiphilic copolymers is generally preferred for obtaining a protective hydrophilic cloud on nanoparticles, as it avoids the possibility of rapid coating desorption upon dilution or after contact with blood components. This approach has been employed with poly(lactic acid) (PLA), poly(caprolactone) and poly(cyanoacrylate) polymers, which were chemically coupled to PEG [32,43–45]. Although these copolymers have not been used until now as nanoparticles in cancer chemotherapy, it is likely that this type of construction will be extensively investigated in the near future.

Another approach worth mentioning consists in tumor targeting with long-circulating carrier followed by irradiation of tumor site in case of photodynamic therapy (PDT) [46]. This combination was aimed at improving anticancer efficacy in an EMT-6 tumor-bearing mice model, while better avoiding systemic phototoxicity. Unfortunately, although the idea was clever, this system consisting in PLA nanospheres covered by adsorbed PEG did not allow a higher intratumoral accumulation of its incorporated photosensitizer (hexadecafluoro zinc phthalocyanine) [46]. The fragility of the adsorbed coating and the large particle size (>900 nm) could be

responsible for this failure. However, formulation of the photosensitizer in the biodegradable nanospheres improved PDT response (by possibly influencing the intratumoral distribution pattern of the photosensitizer), while providing prolonged tumor sensitivity towards PDT [46]. Finally, one should expect a lower systemic phototoxicity, as the carrier system successfully increased the tumor/skin and tumor/muscle uptake ratios for the drug.

2.2. Local administration: subcutaneous or intratumoral

Unlike water-soluble molecules, which are rapidly absorbed through the blood capillary wall and pass into circulation, small particles injected locally infiltrate into the interstitial space around the injection site and are gradually absorbed by the lymphatic capillaries into the lymphatic system [47,48].

For that reason, subcutaneously or locally injected (in the peri-tumoral region) nanoparticles can be used for lymphatic targeting, i.e. as a tool for chemotherapy against lymphatic tumors or metastases. For example, aclarubicin adsorbed onto activated carbon particles was tested after subcutaneous injection in mice, against a murine model (P388 leukemia cells) of lymph node metastases [49]. The same system was also used in patients after intratumoral and peritumoral injections, as a locoregional chemotherapy adjuvant for breast cancer [50]. In both applications, this carrier system distributed selectively high levels of free aclarubicin to the regional lymphatic system and low levels to the rest of the body [49,50]. However, this carrier system is open to criticism, as it is not biodegradable, and as it is rather big (>100 nm), impeding the drainage from the injection site through the aqueous channels [47]. Besides, the drug is associated to the particles by adsorption, leading to a rapid release with possible systemic absorption.

It would seem that PIBCA nanocapsules with an oily core for hydrophobic drugs [48] or biodegradable systems coated by adsorption of the surfactant Poloxamine 904 [47] offer interesting properties for future investigations in this field. Indeed, PIBCA nanocapsules, as compared to liposome or emulsion formulations, showed a potential to retain the lipophilic indicator 12-(9-anthroxy)stearic acid (ASA) in the regional lymph nodes during 168 h after intramuscular administration [48]. On the same way, Poloxamine 904 caused an increased sequestration of the particles in lymph nodes, which would probably reduce systemic absorption of any encapsulated drugs [47]. Other systems regarded as promising in the fight against cancer are ultrasmall superparamagnetic iron oxides particles (USPIO) with an anticancer drug entrapped in their ferrous core (see below).

A study combining intratumoral administration of gadolinium-loaded chitosan nanoparticles (gadopentetic acid–chitosan complex nanoparticles) and neutron-capture therapy was performed on the B16F10 melanoma model subcutaneously implanted in mice [51]. Results showed an outstanding gadolinium retention in tumor tissue when it was encapsulated, with respect to the free drug (a highly water-soluble compound with a rapid elimination). Irradiation of the tumors was performed 8 h after the last intratumoral injection of gadolinium nanoparticles and prevented further tumor growth in the animals treated and increased their life expectancy [51].

3. Nanoparticles for tumor cells targeting and delivery

3.1. Delivery of antisense oligonucleotides (ODNs) to cells

After intratumoral injection, the bioavailability of ODNs is seriously reduced due to their fast degradation by ubiquitous exo- and endonucleases [52]. Moreover, their negative charge seriously hinders the intracellular penetration of theses short fragments of nucleic acids [52]. This leads to a somewhat reduced therapeutic efficacy. In order to prevent the ODN degradation and improve their intracellular capture, it was proposed to associate them with nanoparticles [53–55].

In vitro growth inhibition was carried out on human tumorigenic cells (HBL100ras1, a clone obtained from the human mammary cell line HBL100) using anti-ras ODN-loaded PIHCA nanospheres [56]. In this study, the carrier system consisted of a cationic hydrophobic detergent (hexadecyltrimethylammoniumbromide (CTAB)), which interacted with the ODN by ion-pairing. This hydro-

phobic complex was then adsorbed onto the hydrophobic and negatively charged surfaces of cyanoacrylate nanospheres [56].

In vivo, HBL100ras1 cells implanted in nude mice were treated intratumorally with several formulations of ODNs targeted against Ha-ras oncogene. Tumor growth inhibition was achieved at concentrations a 100 times lower than those needed with free ODN, when the ODN–CTAB complex was adsorbed onto the surface of the PIHCA nanospheres. Interestingly, the ODN–CTAB complex alone did not exert any effect on HBL100ras1 cells proliferation. This clearly demonstrates the need for both components (CTAB cations and PIHCA nanospheres) in order to achieve the biological effect of the ODN [56].

This sine qua non condition for an anticancer effect to materialize could be explained by the ODN intracellular stability and the controlled release provided by the nanoparticles. Indeed, the analysis of the amount of intact intracellular ODN in cell culture experiments revealed concentrations 100-fold higher in the cells treated with ODN–CTAB adsorbed onto nanospheres [56]. On top of it, the cellular uptake of ODN is higher when ODN is associated with nanoparticles [57].

In short, the nanospheres were able to enhance ODN cell internalization and to protect ODN from rapid intracellular breakdown, which led to a considerably higher intracellular concentration of intact ODN and to a more efficient antisense activity.

On the other hand, the ODN release was followed by the release of the detergent CTAB, which, at high intracellular concentrations, could induce cell toxicity. Cholesterol-modified ODNs, capable of direct adsorption onto poly(alkylcyanoacrylate) nanospheres without the need for potentially toxic intermediates, have also been tested, but they proved less able to inhibit T24 human bladder carcinoma cells proliferation in culture than the system previously described [58].

To circumvent CTAB toxicity while maintaining an efficient biological activity, another polymeric vehicle was developed. Functional nanospheres were obtained by free radical emulsion polymerization of methylmethacrylate using quaternary ammonium salt of 2-(dimethylamino)ethyl methacrylate as the reactive emulsifier [59]. C-myb antisense ODN successfully associated with the core shell nanospheres through a reversible ionic interaction with the positively charged ammonia at the particles surface. This carrier system was tested in vitro, on HL60 leukemia cells. Long and effective inhibition of cells growth was observed, occurring through an antisense mechanism. This was attributed to the particulate form of ODN, which, relative to free ODN, demonstrated the following advantages: (i) an increased ODN cellular uptake, probably through endocytosis, (ii) a diminished ODN degradation, resulting from their enhanced protection against nucleases, (iii) a prolonged half-life inside cells, due to the continuous release of intact ODN, which was maintained for up to 8 days of culture [59]. Unfortunately, this carrier system can not be administered in vivo as it is not biodegradable.

Another approach developed to associate ODNs to nanospheres by formation of an ion pair at the surface was to replace CTAB by the cationic dextran derivative diethylaminoethyl-dextran, which associated with PIHCA nanospheres when dissolved in the polymerization medium [60]. However, no study was performed on cancerous cells with this carrier.

In all these systems, ODNs were adsorbed onto the surface of the nanoparticles by electrostatic interactions. In protein rich biological media (cell culture medium or in vivo), rapid release of ODN may occur. Moreover, ionic binding may induce ODNs conformation alterations, which make them unlikely to hybridize with their target [61].

In order to completely shield ODNs from nucleases attack, a new system based on PIHCA nanocapsules has recently been conceived [55].

These nanocapsules, which feature an aqueous core containing oligonucleotides, were prepared by interfacial polymerization of isobutylcyanoacrylate in a W/O emulsion. Ultracentrifugation and resuspension in water yielded a dispersion of these water core containing nanocapsules, with a size ranging between 20 and 400 nm. ODNs loading did not significantly influence zeta potential, suggesting that they were located within the core of the nanocapsules. Fluorescence quenching assays confirmed that fluorescent ODNs were located in the aqueous core of the nanocapsules, surrounded by a polymeric wall, rendering them inaccessible to the quencher [55]. On the other hand, when fluorescent ODNs were free in solution, fluorophores were highly accessible and significant quenching did occur. Similar quenching

could be obtained with nanoencapsulated ODNs only after performing the hydrolysis of the nanocapsule polymer wall, thus releasing the ODNs [55].

Further studies also demonstrated that nanoencapsulation was able to protect ODNs against degradation by serum nucleases contained in the cell culture medium. This protection was even more efficient than that obtained with CTAB-coated nanospheres, which, as mentioned earlier, only allowed simple adsorption of ODNs onto the surface rather than encapsulation [55].

For in vivo experiments, phosphorothioate ODNs against EWS Fli-1 chimeric RNA were encapsulated into the cyanoacrylate nanocapsules and their efficacy was tested in vivo on experimental Ewing sarcoma, after intratumoral administration [62]. As shown in Fig. 3, only intratumoral injections of ODN-loaded nanocapsules led to a significant inhibition of tumor growth at a cumulative dose of 14.4 nmol. Furthermore, no antisense effect could be detected with the ODN free [62]. In a previous study, other authors showed that a cumulative dose of 500 nmol ODN was needed to observe an inhibition of tumor growth in a similar model [63]. Not only did the use of nanocapsules make it possible to obtain a tumor growth inhibition comparable to that observed by these authors [63], but the administered doses were as much as 35 times lower. This may allow to use lower phosphorothioate doses, which in turn would reduce the loss of specificity, to which phosphorothioates are subject at high doses, hence rendering them less toxic [62]. Understanding how ODN nanocapsules led to more efficient tumor growth inhibition is relatively easy: first, the encapsulation of ODNs provided an enhanced protection against in vivo degradation, resulting in a higher number of ODNs available to fight the tumor; second, the nanocapsules may act as a controlled release system, leading to a higher ratio of ODNs targeting the tumor cells [55,62].

Consequently, the use of phosphorothioates at low doses combined with conventional nanocapsules may represent a new and safe solution for the administration of antisense therapy in vivo.

Finally, all the described ODNs associated to nanoparticles are believed to enter the tumoral cells by endocytosis. As all the studied ODNs had a specific therapeutic efficacy, this means that the ODNs delivered by nanoparticles escape from the lysosome compartment before degradation. How they do so, remains unclear. Some authors attributed

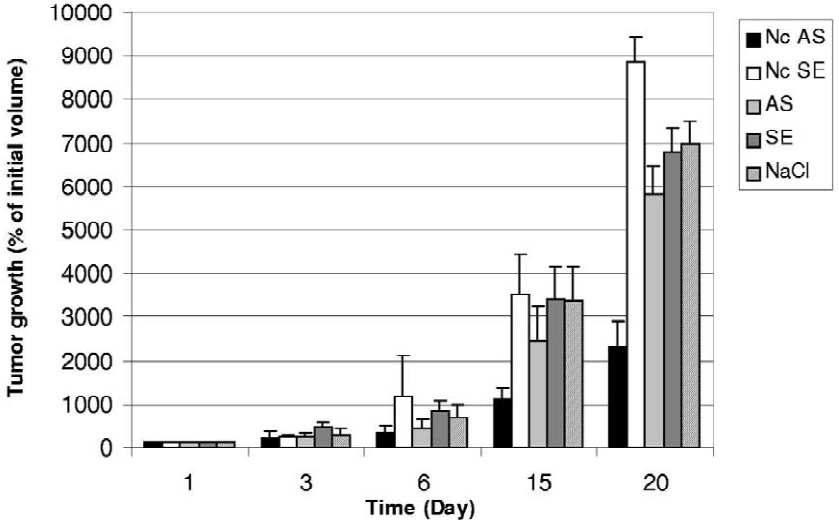


Fig. 3. Effect during 20 days of antisense ODN (ODN against EWS Fli-1 chimeric RNA) on Ewing sarcoma growth in nude mice, expressed in % of day 1 tumor volume (100%). Eight intratumoral injections were performed at day 1, 3, 6, 8, 10, 13, 15, 17. All ODNs, free or encapsulated, were used at a dose of 1.6 nmol for each injection (I.E. at a cumulative dose of 14.4 nmol). **NC AS:** antisense ODN in nanocapsules; **NC SE:** sense ODN in nanocapsules; **AS:** free antisense ODN; **SE:** free sense ODN; **NaCl:** vehicle (NaCl 0.9 g/l) (adapted from Ref. [62]).

it to the cyanoacrylate polymer and/or its degradation products [55]; this would be unlikely, since this biological activity was also demonstrated with the non-biodegradable poly(methylmethacrylate) nanospheres. Hence this question deserves further investigation.

A further step would be to modify the nanocapsules surface with hydrophilic polymers like PEG or functionalized PEG. Surface modification would allow for a more convenient systemic administration (i.e. intravenously), by providing a wider spectrum of pathological sites that could be targeted by such a carrier (for example, not MPS-localized tumors).

3.2. Reversion of multidrug resistance in tumor cells

Anticancer drugs, even if they are located in the tumoral interstitium, can turn out to be of limited efficacy against numerous solid tumor types, especially because cancer cells are able to develop mechanisms of resistance [9,10]. These mechanisms allow tumors to evade chemotherapy. For example, multidrug resistance (MDR) is one of the most important problem in chemotherapy. MDR is mainly due to the overexpression of the plasma membrane P-glycoprotein (Pgp), which is capable of extruding various generally positively charged xenobiotics, including some anticancer drugs, out of the cell [9,10]. However, as MDR is always multifactorial, other mechanisms are associated with this drug efflux pump in cancer cells, such as enzymatic function modification (topoisomerase, glutathione *S*-transferase) or altered intracellular drug distribution due to increased drug sequestration into cytoplasmic acidic vesicles [10,64]. This later point leads finally to an extrusion of the sequestered drug into the external medium and this reduces also drug–target interaction, as this confinement preclude any contact of the cytotoxic drug with the common target DNA or nuclear enzymes [64].

In order to restore the tumoral cell sensitivity to anticancer drugs by circumventing Pgp-mediated MDR, several strategies have been applied, including the co-administration of Pgp reversing agents (verapamil, amiodarone, cyclosporine) or the encapsulation of antitumoral drugs in colloidal carriers (liposomes, nanoparticles) [10]. The rationale behind the association of drugs with colloidal carriers against drug resistance comes from the fact that Pgp probably recognizes the drug to be effluxed out of the tumoral cell only when this drug is present in the plasma membrane, and not when it is located in the cytoplasm or lysosomes, after endocytosis [64,65].

As many tumor cells are resistant to doxorubicin, which is a Pgp substrate, the incorporation of this compound into biodegradable polycyanoacrylate nanospheres has been investigated in resistant cell lines.

In an in vitro model of doxorubicin-resistant rat glioblastoma (C6 cells sublines), doxorubicin incorporated into PIHCA nanospheres were always more cytotoxic than the free drug, whereas, surprisingly, intracellular concentration of doxorubicin was always lower with nanoparticles than with the free drug [65]. The role played by the polymer constituting the nanospheres (intact nanospheres or degradation product) in inhibiting Pgp by a direct interaction with the protein could be excluded [66]. It was also observed on C6 cell sublines with different expression of Pgp that doxorubicin nanospheres were only efficient on pure Pgp-mediated MDR phenotype cells and not on the additional mechanisms of resistance to doxorubicin (for example: alteration of topoisomerase II, drug detoxification, etc.) [65].

Opposite results were obtained with doxorubicin incorporated into the more rapidly biodegraded PIBCA nanospheres (compared to the former PIHCA nanospheres) and tested in P388/ADR cell lines (P388/ADR, a resistant murine leukemia subline overexpressing Pgp) [67]. It was observed that: (i) cellular uptake was higher when doxorubicin was loaded into the nanospheres, (ii) cell uptake kinetics of doxorubicin nanoparticles was unchanged in the presence of cytochalasin B, an endocytosis inhibitor, (iii) efflux studies showed a similar profile for doxorubicin in nanoparticulate or free form. This suggests that PIBCA nanospheres did not enter the cells (in contrast to a previous hypothesis [68]). Thus, the mechanism of Pgp reversion by nanoparticles could only be explained by a local delivery of the drug in high concentration close to the cell membrane, after degradation of the polymeric carrier. Such local microconcentration of doxorubicin was supposed to be able to saturate Pgp [67].

Hence, the mechanism to overcome Pgp-mediated MDR with PIBCA and PIHCA doxorubicin-loaded

nanospheres was found not only to be related to the adsorption of nanoparticles to the cell surface, but also to an increased diffusion of doxorubicin across the plasma membrane, thanks to the formation of an ion pair between the negatively charged cyanoacrylic acid (a nanoparticles degradation product) and the positively charged doxorubicin [69]. Such an ion-pair formation has been evidenced by Raman spectroscopy and by ion-pair reversed-phase HPLC [69,70]. At the same time, supramolecular structure appeared to be globally more hydrophobic, which could facilitate diffusion across the cell membrane [70] (Fig. 4).

Such a complex mechanism for overcoming Pgp-mediated MDR was only observed with cyanoacrylate type nanoparticles. Indeed, to be effective, three criteria need to be fulfilled: (1) the particulate structure should adhere to the cell membrane, providing a concentration gradient, (2) the drug release and nanoparticles degradation should occur simultaneously, and (3) an ion pair should form, in order to mask the positive charge of the drug. The failure to overcome Pgp-mediated MDR resistance with nanoparticles designed with other polymers could be explained by an inappropriate release mechanism of drug (diffusion, for instance, could lead to the release of the active compound without the polymeric counter ion), by a too slow degradation kinetics of the polymer, or by the size of the polymeric counter ion, which in the case of polylactic acid, for example, could be the limiting factor for diffusion across cell membrane and therapeutic activity.

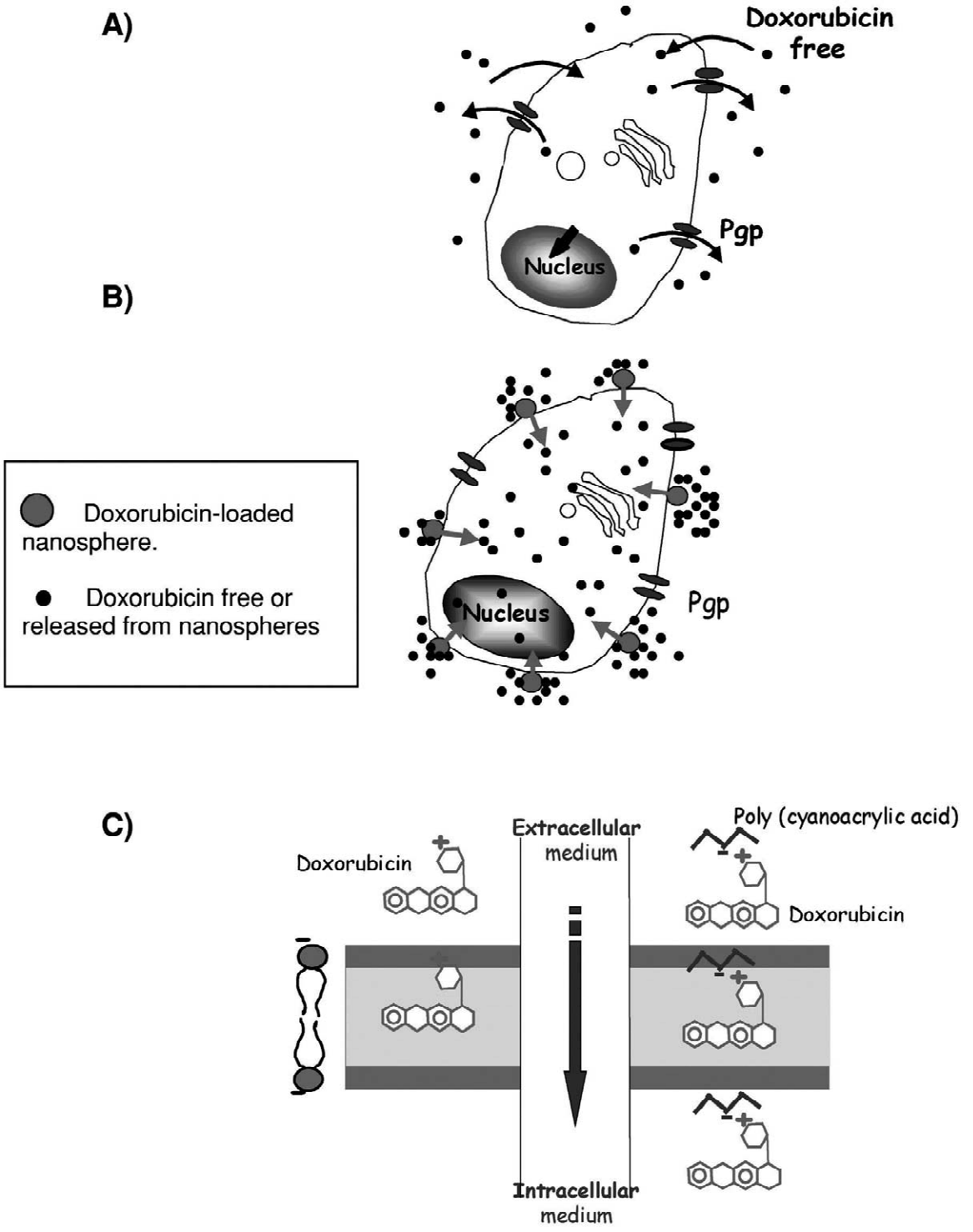
When doxorubicin was coupled via an ionic interaction to non-biodegradable polymethacrylate nanospheres (between the amino sugar of the anticancer drug and the methacrylate acid residue on the nanospheres surface), the carrier system's MDR (Pgp-mediated) reversal differed from that of doxorubicin incorporated into biodegradable cyanoacrylate polymers.

First, when adsorbed onto the surface of poly(methacrylate) nanospheres, doxorubicin was demonstrated to be cell internalized by an endocytotic process in cultured rat hepatocytes and in U-937 cells (human monocyte-like cancer cell line expressing a Pgp) [71]. Then, once internalized, polymethacrylate nanospheres generated an intracellular sustained release of doxorubicin in U-937 cells. The consequence of this controlled release was a slower efflux of doxorubicin. As a result, a higher intracellular accumulation, related to a more important cytotoxicity on U-937 cells, was noted for encapsulated doxorubicin than for free doxorubicin [71]. However, such a carrier, despite its ability to mask the positive charge of doxorubicin, is of limited use in vivo, since it is not biodegradable.

Masking the positive charge of the amino sugar of doxorubicin appears to be a key point to overcome Pgp-mediated MDR. At the same time, the cytotoxic activity of doxorubicin was only slowly compromised after chemical modifications of the amino sugar [72]. Consequently, some studies focused on developing systems featuring a covalent linkage between the polymers and the amino sugar of doxorubicin.

In one instance, doxorubicin was covalently anchored to a poly(ethylene glycol) (molecular weight: 14 400 and 3500, respectively)–poly(aspartic acid) block copolymer before micelles formation [73]. In an other instance, doxorubicin was incorporated into gelatin nanospheres by a covalent bond on its amino group via glutaraldehyde [74]. Unfortunately, both investigations showed no or only marginal antitumor activity against a C26 tumor (mouse colon adenocarcinoma) in vivo, and in certain cases an increased doxorubicin cardiotoxicity was even noted. Once again this lack of antitumor activity may be due to the slow dissociation of the complex due to the covalent linkage or to the slow diffusion of the complex across cellular membrane (the bigger the complex the slower the diffusion).

Fig. 4. Doxorubicin acting on a resistant tumor cell, expressing Pgp. (A) Free doxorubicin (as positively charged) enters the tumor cell by diffusion but is effluxed by Pgp, resulting in the absence of therapeutic efficacy. (B) Doxorubicin-loaded poly(cyanoacrylate) nanospheres adhere at the tumor cell membrane where they release their drug content, resulting in microconcentration gradient of doxorubicin at the cell membrane, which could saturate Pgp and reverse MDR. (C) Doxorubicin forms an ion pair with the degradation product of the poly(cyanoacrylate) nanospheres (i.e. cyanoacrylic acid). As the positive charge of doxorubicin is masked (rendering the compound more hydrophobic), doxorubicin cell internalization by diffusion is facilitated. Moreover, Pgp-mediated MDR resistance is bypassed, as 'neutralized' doxorubicin is no more effluxed by Pgp, while being still active.


A)
Doxorubicin free
Pgp
Nucleus
B)
Doxorubicin-loaded nanosphere.
Doxorubicin free or released from nanospheres
Nucleus
Pgp
C)
Doxorubicin
Extracellular medium
Poly (cyanoacrylic acid)
Doxorubicin
Intracellular medium

It is now clear that conventional poly-(cyanoacrylate) nanoparticles allow to overcome doxorubicin Pgp-mediated MDR in vitro, only when there is a close contact between the nanoparticles and the resistant cancer cell lines [69]. Furthermore, preliminary in vivo studies exhibited promising results on MDR tumors, but this effect was not considered efficient enough to elicit a clear positive response, especially because the tumors cells were grafted intraperitoneally and the nanoparticles administered in the same cavity. Such a model may be considered as not very relevant for clinical applications [66]. Thus, the in vivo efficacy of doxorubicin-loaded nanoparticles is questionable. Therefore, other strategies to bypass MDR, such as the use of Stealth™ poly(cyanoacrylate) nanoparticles also for MPS localized tumors which allow a direct contact with the neoplastic cells, could be considered, as well as the co-administration of doxorubicin with chemo-sensitizing agents, generally acting as Pgp inhibitors.

Indeed, when MDR-reversing agents like amiodarone or verapamil were added to the cell culture medium, in combination with doxorubicin incorporated into PIBCA or PIHCA nanospheres, the cytotoxicity of the anticancer agent on a resistant DC3F AD/AZA subline (Chinese hamster lung cell line) was still improved [75]. Unfortunately, the doses at which these membrane modulators exerted an MDR reversion were not compatible with human administration [75].

This is the reason why co-encapsulation of the reversing agent cyclosporin A and the anticancer drug doxorubicin into PIBCA nanospheres was experimented [76]. This approach strove to reduce the side-effects of both drugs while enhancing their efficacy. This formulation, compared to other treatments (incubation of cyclosporin + doxorubicin, or doxorubicin nanoparticles + cyclosporin), elicited the most effective growth rate inhibition on P388/ADR cells. Such a high efficacy was supposed to result from the synergistic effect due to the rapid release of both doxorubicin and cyclosporin at the surface of the cancerous cell, allowing a better internalization of doxorubicin, while inhibiting its efflux by blocking the Pgp with cyclosporin [76]. This highlights the importance of a similar release of both drugs from the nanospheres to bypass Pgp-mediated MDR. Polyalkylcyanoacrylate nanoparticles mainly release their drug content by biodegradation [77], thus rendering the release profile of an entrapped compound independent of its physicochemical characteristics, which could make them the carrier of choice for such strategies.

3.3. Molecular addressing of nanoparticles ('active targeting')

A lot of effort has been devoted to achieving 'active targeting', in order to deliver drugs to the right cells, based on molecular recognition processes (ligand–receptor or antibody–antigene interactions). Moreover, in some cases, active targeting may lead to receptor-mediated cell internalization.

For example, by taking advantage of the over-expression of folate receptors on the surface of malignant human cells, folate-conjugated nanoparticles were developed, in hope that their folate grafting would help them actively and specifically target cancer cells [78]. Interestingly, surface plasmon resonance revealed that folate grafted to PEGylated cyanoacrylate nanoparticles had a 10-fold higher apparent affinity for the folate-binding protein (FBP) than free folate did. Indeed, the particles represent a multivalent form of the ligand folic acid, and folate receptors are often disposed in clusters. As a result, conjugated nanoparticles could display a multivalent and hence stronger interaction with the surface of the malignant cells [78]. Moreover, confocal microscopy demonstrated that folate nanoparticles, comparatively to non conjugated nanoparticles, were selectively taken up by the folate receptor-bearing KB3-1 cells, but not by the MCF-7 cells, devoid of folate receptor. In the former case, the folate nanoparticles were found to be localized in the cell cytoplasm, as a consequence of folate receptor-mediated endocytosis [79].

So not only do folate nanoparticles selectively target cancer cells, but they also improve the internalization of the encapsulated drugs within the targeted cancer cells.

4. Nanoparticles as tumor biomarkers

4.1. In vitro

Nanoparticles can be used for qualitative or quan-

titative in vitro detection of tumor cells. They help the detection process by concentrating and protecting a marker from degradation, in order to render the analysis more sensitive.

For example, streptavidin-coated fluorescent polystyrene nanospheres (Fluospheres® (green fluorescence) and TransFluospheres® (red fluorescence)) were used in single color flow cytometry to detect the epidermal growth factor receptor (EGFR) on A431 cells (human epidermoid carcinoma cells) [80]. The results showed that the fluorescent nanospheres provided a sensitivity 25-fold that of the conjugate streptavidin–fluorescein. The encapsulation of fluorescent markers resulted in objects that were brighter and more concentrated than when simple conjugates of single dyes were used. Moreover, the fluorescent nanoparticles were used in combination with R-Phycoerythrin (R-PE, reagent for flow cytometry) in multicolor flow cytometry, enabling the concomitant detection of the CD3 and CD4 receptors on JURKAT cells (human acute T-cell leukemia cells) [80]. However, although specific and sensitive, these systems could not be used for precise quantitative analysis.

Another approach was the encapsulation of inorganic biomarkers, rather than fluorescent organic markers. These compounds are more photostable and not hampered by the intrinsic fluorescence (background signal) emitted by cells and tissues, which makes them more suitable and sensitive for qualitative and especially quantitative detection. For example, streptavidin-coated nanoparticles containing lanthanide chelates were used in the quantitative immunohistochemistry analysis with time-resolved fluorescence (TRF) imaging [81]. Both the amount of nanoparticles detected and the specific signal measured from a TRF image correlated linearly with the amount of antigen (prostate-specific antigen) used [81]. Quantitative measurement is thus possible.

Another study of inorganic luminescent dyes experimented nanoparticles composed of Rubpy doped in a silica network, for qualitative in vitro use [82]. In this assay, lauroyl groups were grafted at the nanoparticles surface to stain human leukemia cells. The mechanism was based on the ability of the hydrophobic moiety (lauroyl group) anchored to the nanoparticles to penetrate the cell membranes [82]. This interaction, which is although not specific to tumoral cells, can be improved by grafting biomolecules like antibodies on nanoparticles surface to provide an accurate in vitro neoplastic cell detection.

4.2. In vivo

Contrast agents have been loaded onto nanoparticles for tumor diagnosis purposes. As previously described for chemotherapy, the physico-chemical characteristics (particle size, surface charge, surface coating, stability) of the nanoparticles allow the redirection and the concentration of the marker at the site of interest.

Labelled colloidal particles could be used as radiodiagnostic agents. On the other hand, some nonlabelled colloidal systems are already in use and some are still being tested as contrast agents in related diagnosis procedures such as computed tomography and NMR imaging.

4.2.1. Radiodiagnosis (scintigraphy)

To our knowledge, a study of radionucleides used in diagnostic imaging with nanoparticles for cancer detection has not yet been published. However, as conventional colloidal particles can be phagocytosed by the liver, the spleen, the lungs and the bone marrow and as long-circulating nanoparticles can have a compartmental localization in the blood circulation or the lymphatic system—all these organs being potential sites for tumor development, these colloidal systems could potentially improve tumor diagnosis.

Examples of such systems are ^{99m}Tc-labelled colloids, particularly sulfur colloids, antimony sulfite colloids, as well as denaturated human serum albumin particles [83]. A different study used polycyanoacrylate nanoparticles with diethyltriaminepentaacetic acid (DTPA) as a spacer in order to fix the isotopes ^{111}In or ^{99m}Tc. This system was relatively stable during the whole period of investigation and showed an accumulation in the MPS organs of rabbits and also of men [84].

4.2.2. Computed tomography

Indirect computed tomography was performed, after subcutaneous injection of iodinated nanoparticles to swine, in order to detect cancerous lymph nodes in a cutaneous melanoma model [85,86]. This particulate contrast agent provided adequate

lymphotropy (the particles were phagocytosed by macrophages within targeted lymph nodes) leading to a high contrast resolution. The increased conspicuity induced by the iodinated particles permitted: (i) to detect more deeply seated, traditionally inaccessible lymph nodes, (ii) to visualize alterations in lymph nodes internal architecture, and (iii) to elucidate the lymphatic drainage patterns. However, this carrier system behaved poorly when precise quantitative determinations were needed. Indeed, it could not differentiate nodes with 25% or less tumor replacement, from normal nodes [85,86].

4.2.3. Magnetic resonance imaging (MRI)

Superparamagnetic nanoparticles were used as contrast agent in magnetic resonance imaging. They consisted of an inorganic core of iron oxide (magnetite Fe_3O_4, maghemite or other insoluble ferrites) coated or not with polymers like dextran [87]. These nanoparticles were classified in two main groups, according to their size, which can affect their plasma half-life, their biodistribution and thus their field of application [87]. These groups were labelled:

- SPIOs (superparamagnetic iron oxides), whose nanoparticles had a size greater than 50 nm (coating included)—these devices can be related to the conventional nanoparticles described previously
- USPIOs (ultrasmall superparamagnetic iron oxides) or LCDIO (see below), whose nanoparticles were smaller than 50 nm—these devices can be related to the long-circulating nanoparticles described previously

Lumirem® (silicon-coated iron oxide particles with a diameter of 300 nm) and Endorem® (magnetite nanoparticles of 150 nm in diameter, coated with dextran) are commercial names of SPIOs available on the market [87]. These nanoparticulate contrast agents are, respectively, used for gastro-intestinal tract imaging and for liver and spleen diseases detection [87]. In the case of Endorem®, the massive uptake of the nanoparticles by Kupffer cells allowed to increase the contrast between the healthy and the diseased tissue, like tumors or metastases, devoid of Kupffer cells [87].

Sinerem® (magnetite nanoparticles of 30 nm in diameter, coated with dextran) is an example of USPIO on the market [87]. Due to their long-circulating properties, USPIOs can be used for blood pool and tumor imaging (experimental imaging), based upon the detection and characterization of the lesions by their vascular appearance [87].

For cancer diagnosis, discrimination between the tumor and the surrounding normal, but often oedematous tissue, depends in part on the neovascularization of the tumor, which is needed for the accumulation of the contrast agent to occur in the interstitium. Moreover, for accurate anatomic definition, the agent must remain localized within the boundaries of the neoplastic tissue during the time required to obtain the images.

Long-circulating dextran-coated iron oxide (LCDIO) particles were investigated in vivo, in rat malignant brain neoplasms [88]. The LCDIO particles accumulated in the intracerebrally implanted tumors (9L murine gliosarcoma, C6 murine glioma) and were mainly internalized by endocytosis in tumor cells [88,89]. They were also taken up to a lesser extent by the tumor infiltrating macrophages and by endothelial cells in the areas of active angiogenesis [88–90]. The total amount of LCDIO taken up by the glioma was sufficient to alter the MR signal intensity [88,89]. The results obtained with these models suggested that an improved delineation of tumor margins is possible with iron oxide nanoparticles, because of their low diffusivity and their endocytosis by metabolically active cells (cancer cells, as well as tumor infiltrating macrophages) [88,89]. Interestingly, micrometastases located some distance from the main tumor margin also showed particles uptake and could thus be visualized by MR imaging [89].

Sinerem® was also used for the detection of human brain tumors [91]. Again, USPIOs permitted a precise and prolonged delineation of the brain tumor margin. This constituted a major improvement on the performance of gadolinium chelate, another contrast agent, which diffused into normal surrounding brain tissue, causing progressive blurring of the tumors margins [91]. These improvements obtained by using nanoparticles have favorable implications for diagnosis biopsies and planning of surgical resections.

Finally, MR imaging may also become an investigation tool in the study of tumoral growth kinetics in vivo, since a positive correlation has been

observed between LCDIO uptake by tumor cells and tumor doubling time [88].

Moreover, due to their small size, USPIOs can also be used for lymph node diseases detection (ongoing clinical trials), after intravenous, subcutaneous or intramuscular injection [87]. For this application, the crucial point in designing lymphotrophic contrast agents is to minimize their recognition by the liver and spleen macrophages, while still keeping them recognizable by the lymph node macrophages.

Sinerem® has been used for lymphography of hyperplastic or metastatic (metastasis of a nickel-induced rabdomyosarcoma) lymph nodes in rats, after intravenous administration [87]. Another USPIO device consisting of monocrystalline iron oxide nanoparticles (MION-46, a nanoparticulate contrast agent designed specially for lymphography, coated by an extended dextran layer, with a hydrodynamic diameter of 20 nm) was also tested in rats and rabbits to detect lymph node metastases (metastasis of VX2 carcinoma) and tumor-associated lymph node hyperplasia, using different administration routes (subcutaneous, intravenous and intraarterially) [92]. In both assays, hyperplastic lymph nodes showed MR images consistent with an active uptake and a clustering of the nanoparticles inside the macrophages of lymphatic sinuses [92,93]. The differentiation of malignant and normal lymph nodes was sometimes hindered by the following two problems: first, the Sinerem® nanoparticles showed a slight tumor accumulation, due to diffusive extravasation across the tumoral hyperpermeable vasculature [93]. So much so, that in some extreme cases, the tumors would not be able to be differentiated from the healthy lymphatics, as they both take up (even though by different mechanisms) the contrast agent. Second, when the MION-46 formulation was used, there was a lack of contrast agent accumulation in marginal follicles of normal lymph nodes, which, on occasion, rendered them indistinguishable from nodes with micrometastases [92]. Despite these two problems, which should be kept in mind during diagnosis, USPIOs appear to be very efficient tools for MR imaging.

It is also important to discuss how safe these ferrous nanoparticles are, when applied to MR diagnosis. Intralysosomal iron oxides are known to be metabolized into elemental iron and oxygen by the hydrolytic enzymes, whereupon the component iron subsequently joins normal body stores. Moreover, as iron homeostasis is carefully controlled by absorption, excretion and storage, it is expected that after the administration of ferrous nanoparticles, the iron intestinal uptake would temporarily be downregulated and that the iron excretion would be increased. On the other hand, acute toxicity is rarely of concern, with regard to the administered doses and the high short-time safety index of iron oxides. Chronic toxicity, however, may be of concern if high doses of iron oxides are administered repeatedly [92].

Finally, if superparamagnetic nanoparticles were endowed with the capability of specific recognition of cell types, a more precise localization of selected cells could be achieved. Such an approach has been reported using MIONs covered with arabinogalactane, which are particularly sensitive to the asialoglycoprotein receptors present on normal hepatocytes [87]. Moreover, as the generally used dextran coat offers many available hydroxyl groups, these targets could be improved by grafting molecules like transferrin (by partial oxidation of dextran) [94]. One could imagine the coupling of other receptor-directed molecules, such as monoclonal antibodies, EGF, VEGF and so on. Another strategy would consist in replacing the dextran coating by other materials, like peptides, which also provide an ideal platform for the attachment of biological modifiers [95].

5. Conclusion

Most solid tumors possess unique pathophysiological characteristics that are not observed in normal tissues or organs, such as extensive angiogenesis and hence hypervasculature, defective vascular architecture, impaired lymphatic drainage, and greatly increased production of a number of permeability mediators.

This review presented how nanoparticles took advantage of these special features and how nanoparticles could act as a vehicle to specifically deliver cancer-fighting drugs to tumors.

Nanoparticles could do so by an indirect mecha-

nism consisting in targeting the tissue adjacent to the tumor (i.e. Kupffer cells in the liver), which in turn can act as a drug reservoir in the fight against the neighboring neoplastic cells.

Direct targeting of tumor tissues by nanoparticles is also possible when one takes advantage of the tumor vasculature hyperpermeability. This approach should provide the previously described EPR effect, but has yet to be clearly demonstrated for nanoparticles.

However, the use of either targeting methods does result in an increased efficacy of anticancer drugs; a reduction of their side effects was also generally observed.

Very encouraging results were obtained at the cellular level by using nanoparticles: efficient drug protection, cell internalization, controlled release, or reversion of the MDR resistance could be demonstrated. Poly(alkylcyanoacrylate) nanoparticles have even reached the status of phase II clinical trials for resistant cancers.

The use of nanoparticles in imaging is also promising, for they allow increased conspicuity and tumor delineation.

The contribution of nanoparticles in cancer chemotherapy will certainly grow, provided that more efficient tumor targeting strategies are developed. Future research will concentrate on active targeting with molecule such as folic acid, as this strategy offers both specific recognition and cell internalization. However, active targeting often entails numerous chemical reactions, as protection/deprotection reactions for instance. The challenge will hence be to avoid a complex synthesis of the constructs.

As far as encapsulation of antitumor drugs within nanoparticles is concerned, it is suspected that future developments will concentrate on emerging molecules acting at the cancerous cell level (taxol, for instance), as well as on drugs acting at the vascular level like angiostatin [96], and tumor necrosis factors [97]. In this later case, there are less biological barriers to overcome, as there is no need for extravasation in the tumoral interstitium, for cell internalization as well as for escaping from the lysosome compartment.

Finally, novel approaches of associating drugs to the nanoparticles should also emerge (prodrugs formulated in nanoparticles, for example) [98].

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