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Development of a nanoparticle-based system for the delivery of retinoic acid into macrophages

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ABSTRACT

The aim of the present work is to prepare nanoparticulate systems that can target and modulate the functions of mononuclear phagocytes by local administration. All-trans retinoic acid (RA) was chosen as an immunomodulator to be encapsulated in biodegradable nanoparticles (NP). Different formulations were prepared by the nanoprecipitation method and poly(D,L)lactic acid based nanocapsules (NC) were selected to continue the study. RA-NC demonstrated a sustained release profile and an enhanced stability for 7 days. The uptake of fluorescent (NileRed) labeled NP was conducted on bone marrow derived macrophages (BMM) *in vitro* and xenograft glioma nude mice *in vivo*. Fluorescent microscopy observations and flow cytometry analysis demonstrated that NR-NC were engulfed by BMM *in vitro* and lasted inside over 7 days. The intratumoral injection of NR-NC confirmed that NC were efficiently uptaken by infiltrated macrophages. The effects of RA loaded NC on BMM were also evaluated by RT^2-PCR array. Our results suggest that polymeric nanoparticles are suitable carriers to deliver RA into macrophages and can offer a new strategy in tumor macrophage-based treatment.

1. Introduction

Macrophages are innate immune cells that play a major role in the physio-pathology of numerous disorders that involve primary or secondary immune mechanisms. In particular, macrophages are crucial to the processes of tissue repair and form an important line of defense against infectious agents. On the other hand, macrophages are considered as playing detrimental functions under several conditions including chronic inflammatory diseases and tumors. Thus, in chronically inflamed tissue, infiltrating macrophages release cytokines/chemokines that lead to a sustained recruitment and activation of immune cells (Chellat et al., 2005). In malignant tumors, the so-called tumor associated macrophages (TAM) are thought to support both tumor progression and metastatic invasion (Coussens and Werb, 2002). Owing to their plasticity and involvement in a large range of pathologies, macrophages are thus considered as potential target in the design of innovative therapies (Ulbrich and Lamprecht, 2010; Watters et al., 2005).

Biodegradable polymeric nanoparticles (NP) are highlighted areas of drug delivery research. They are used to modify the release and distribution profile of active compounds allowing effective delivery to be improved and toxic effects to be lowered (Mora-Huertas et al., 2010). Moreover, polymeric nanoparticles increase intracellular drug delivery and its therapeutic effects *via* enhanced stability and sustained release especially for the drug that act *via* intracytoplasmic receptors. For example, Sahoo and Labhasetwar (2005) reported that NP enhanced paclitaxel efficacy on breast cancer cell line *via* sustained intracellular delivery. Bernardi et al. (2008) also presented the improvement of indomethacin cytotoxic effects on glioma cell line when it was nano-encapsulated. Besides, nanoparticle-based delivery system can be designed to target specific tissues, cells and/or intracellular compartments (Hillaireau and Couvreur, 2009). In this view, it has been long-lastly documented that polymeric nanoparticles without surface modification were preferentially taken up by macrophages following systemic or local administration. The adsorption of serum protein on polymeric-nanoparticle surface (opsonization) is an important factor which allows macrophages to recognize and internalize these particles. The opsonization and phagocytosis of NP are influenced largely by the NP physiochemical properties, in

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particularly the opsonization is increased with the augmented charge (Gessner et al., 2002) and hydrophobicity (Gessner et al., 2000) of NP surface. It was proved that decoration of NP surface with non-ionic and/or hydrophilic groups can decrease opsonization and hence limits phagocytosis (Owens and Peppas, 2006). As a consequence polymeric nanoparticles without surface modification are considered as ideal shuttles to deliver active drugs specifically into macrophages (Chellat et al., 2005; Ulbrich and Lamprecht, 2010). Such a strategy was applied to deliver antileishmanial agents into infected macrophages where nanoparticles allow specific delivery and maintenance of higher drug level inside macrophages (Basu and Lala, 2004).

All-trans retinoic acid (RA), one of the active derivatives of vitamin A, is a ligand of the retinoic acid intracytoplasmic receptors (RAR, RXR) and is known to influence the functions of macrophages. RA can inhibit the macrophage production of inflammatory cytokines and can enhance the secretion of suppressive cytokines as well (Pino-Lagos et al., 2008). It was proven also that RA altered the balance of TH1/TH2 type T cells through its impact on macrophages (Kang et al., 2007). Besides, RA has an influence on macrophages and dendritic cells in tumor. Such, RA inhibit the secretion of the angiogenic factors like VEGF and IL-8 by tumor-activated macrophages (Liss et al., 2002). Darmanin et al. (2007) suggested that RA can improve dendritic cell migration from the tumor to draining lymph nodes and may be boost the antitumor immunity. Poor water solubility and low stability of RA represent the main drawbacks in its dosage form formulations (Szuts and Harosi, 1991). The clinical use of RA was almost associated with rapid decrease of its serum concentration after continuous oral administration or intravenous injection (Achkar et al., 1994). It was documented that the encapsulation of RA can overcome these limitations and therefore offers more advantageous pharmaceutical forms. Ourique et al. (2008) showed that using nanocapsules enhanced RA photostability twofold than RA methanolic solution. Besides, Cirpanli et al. (2005) reported that RA-microspheres had a long chemical stability up to 4 months at 4 °C and lasted release profile over 11 days.

Intratumoral administration appears as an effective method for cancer chemotherapy and immunotherapy that allow to achieve ideal drug concentration in tumors and to limit systemic side effects (Goldberg et al., 2002). Previous reports have demonstrated that nanoparticle intratumoral delivery improves the anti-tumor efficacy. For example, Farokhzad et al. (2006) showed the advantage to use functionalized poly (D,L-lactic-co-glycolic acid)-block-poly(ethyleneglycol) NP in the intratumoral delivery of docetaxel to prostate cancer. Another team reported the enhancement of antitumor efficiency by using methoxy poly(ethylene glycol)-polycaprolactone core–shell NP for the intratumoral administration of cisplatin (Li et al., 2008) and docetaxel (Zheng et al., 2010). Moreover, the use of hyaluronan NP or poly (D,L-lactic-co-glycolic acid) NP for the intratumoral delivery of paclitaxel was also reported (Al-Ghananeem et al., 2009; Sahoo et al., 2004). Among these studies, non-modified surface or polyethyleneglycol (PEG) coated polymeric NP were used to target tumor cells. The PEG chains was utilized to avoid the uptake of intratumoral injected PEG-coated NP by non-tumoral cells. But in the case of polymeric NP without surface modification the uptake of polymeric NP by tumor associated macrophages (TAM) has not been investigated. It was well documented that non-modified surface NP were preferentially taken up by macrophages after opsonization *in vivo* (Owens and Peppas, 2006). So, it can be postulated that following intratumoral injection the polymeric NP could be taken up by TAM.

However, to the best of our knowledge, there is yet no study that attempted to design a NP-based system to deliver an immunologically active molecule into macrophages and, in particular, tumor-associated macrophages. Since in tumors, macrophages are continuously instructed by extracellular signals that shape their behavior, the goal of such a NP-based system would be to support a sustained deviation of macrophage functions that would counterbalance the effects of the macrophage microenvironment.

In this context, our goal was to design NP that meet the following criteria: (i) to be phagocytized by macrophages *in vitro*, (ii) to be phagocytized by tumor-associated macrophages *in vivo*, (iii) to efficiently encapsulate retinoic acid (RA), and (iv) to be biodegradable over a time-period of at least one week.

To achieve these aims, firstly nanospheres (NS) and nanocapsules (NC) formed with different biodegradable polymers (poly(D,L)lactic acid (PLA) and poly(ε-caprolactone) (PCL)) were compared regarding their ability to encapsulate RA. The selected nanoparticle formulation was then labeled by a fluorescent hydrophobic probe, NileRed (NR) to check the ability of such NP to be uptaken *in vitro* by primary cultures of bone marrow derived macrophages (BMM). Afterwards, an *in vivo* model of glioma was chosen to investigate the uptake of locally injected NP by TAM. Finally, the ability of nano-encapsulted RA to deviate the cytokine mRNA profile in bone marrow macrophages was verified.

2. Materials and methods

2.1. Materials

The biodegradable polymers used for NP formulation were poly(D,L)lactic acid (PLA, Mw 20,000 g/mol, Surmodics biomaterial, USA) and poly(ε-caprolactone) (PCL, Mw 14,000 g/mol, Sigma–Aldrich, St. Quentin Fallavier, France). NileRed and all-trans retinoic acid were purchased from Sigma–Aldrich. Montanox® VG 80 and Miglyol® 829 were purchased respectively from Seppic and Condea, France. C57Bl/6 mice (Charles River Laboratories, France) were used as BMM donors. For the tumor xenograft model, Athymic Nude-*Foxn1*^{nu} mice purchased from Harlan (Gannat, France) were used.

2.2. Preparation of NP

Retinoic acid loaded nanoparticles were prepared by using the nanoprecipitation technique (Fessi et al., 1989). Briefly polymer and RA were dissolved in acetone at a concentration of 0.50% and 0.010% (w/w) respectively. To form the core of nanocapsules, organic oil (miglyol® 829 at 0.75%) was added to the solution previously described. This organic phase was then poured into an aqueous phase containing 0.05% of Montanox® VG 80 as surfactant. Nanoparticles (NS or NC) were instantaneously formed by the rapid solvent diffusion inducing polymer precipitation. The nanoparticle suspension was stirred moderately at room temperature for 20 min. Acetone and a part of the water were removed under vacuum using a rotary evaporator (Rotavapor® RE-140, Büchi, Switzerland). Fluorescence labeled NP were prepared in the same way by incorporating NileRed in the organic solution at a concentration of 0.005%. Similar formulas without any active compounds were prepared as controls (blank-NP). The nanoparticle suspensions were stored at 4 °C in hermetically closed vials. Before the *in vivo* application, the NP suspension was centrifuged at 18,000 × *g* for 20 min and the particles were redispersed in a solution of phosphate buffered saline (PBS, 0.1 M, pH 7.4) containing 0.1% of Montanox® VG 80 to obtain a stable suspension with physiological values of pH and osmolarity. The obtained suspension was then sterilized by filtration through 0.2 μm syringe filter (Minisart®, Sartorius, France). All formulations were made in triplicate.