Nano-Emulsions for Biomedical Imaging

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ABSTRACT SUMMARY

This study deals with the development of new generations of contrast agent for biomedical imaging based on nano-emulsions templates. Compared to common and clinical contrast agents, nano-emulsions brings new real advantages like a long circulation in blood, the control of the biodistribution and pharmacokinetics, and the absence of toxicity.

INTRODUCTION

In spite of the progresses of the imagers’ efficiency, notably X-ray and optical modality, their use and potentials are still dramatically limited by the low efficiency and toxicity of contrast agents.1 This study presents the development of new contrast agents overcoming these limitations, based on non-toxic nano-emulsions highly loaded in contrasting materials, intended to fluorescence tomography and/or computed tomography (CT) preclinical imaging. The success of the formulation of such contrast agents relies on several interdependent challenges: (i) Designing efficient and cost-effective contrast that are easy to synthesize and that can be loaded at high concentrations in nanoparticles. (ii) Developing formulations of the contrast agents without organic solvents and specific mechanical device. (iii) Adjusting the nanoparticle surface to allow high stability of the nanoparticles (at least several months), good bioavailability and efficient targeting. (iv) Minimal toxicity of the contrast agent.

EXPERIMENTAL METHODS

2,3,5-Triiodobenzoic acid, α-tocopherol, 4-dimethylaminopyridine, N,N’-dicyclohexylcarbodiimide, dichloromethane, ethyl acetate, cyclohexane were purchased from Sigma Aldrich, France. Non-ionic surfactant (Cremophor ELP®) from BASF (Ludwigshafen, Germany).

The 2,3,5-triiodobenzoic acid (5 g, 0.01 mol), 4-dimethylaminopyridine (0.18 g, 0.0015 mol) and N,N0-dicyclohexylcarbodiimide (2.3 g, 0.011 mol) were sequentially added at room temperature to a solution of DL-a-tocopherol (3.5 g, 0.008 mol) in dichloromethane (250 mL). The reaction mixture was stirred overnight at room temperature and the dicyclohexylurea and other precipitates were removed by filtration. The organic phase was then washed twice with saturated aqueous NaHCO₃, once with saturated NaCl solution and dried with anhydrous Na₂SO₄. The solvent was removed in vacuum and the oil was then purified using cyclohexane and ethyl acetate as an eluent. Reaction yields were around 80%. The resulting product was a light, yellowish viscous oil with a high iodine content of around 41.7%. Nano-emulsions of iodinated α-tocopherol were formulated by the spontaneous nano-emulsification method, as described previously. In short, pure α-tocopheryl 2,3,5-triiodobenzoate (0.75 g) was firstly mixed with the non-ionic hydrophilic surfactant (0.5 g), and maintained at room temperature. Phosphate buffered saline (PBS), used as an aqueous phase (1.88 g), was then added to the surfactant/oil mixture under gentle magnetic stirring. This optimized formulation was chosen to give a compromise between the nano-emulsion size and monodispersity, and the iodine content of the suspension. As a result of the process optimization, this compromise led to a droplet diameter of around 85 nm, with the following formulation parameters: surfactant/oil weight ratio (SOR) 1/4 40%, and (surfactant/oil)/water weight ratio (SOWR) 1/4 40% (see Ref. [2] for details on the formulation process). The α-tocopheryl 2,3,5-triiodobenzoate content in the nano-emulsions (i.e. injectable product) was about 24 wt.%. The schematic represen-
tation of a nano-emulsion droplet is reported in Fig. 1. Finally, nano-emulsions were sterilized by filtration (0.22 mm membrane, Millex-GP, polyethersulfone (PES) membrane, Millipore, Molsheim, France) before intravenous administration.

RESULTS AND DISCUSSION

Contrast agents were formulated as lipid nano-emulsions that consisted in a lipid core, surrounded by a non-ionic surfactant layer (see Fig. 1). The lipid core comprised lipophilic molecules either grafted with iodine compounds for X-ray contrast, and/or solubilized fluorescent dyes with high loading ratio. The surface of the nano-droplets was fully covered by a hydrophilic polymer, like PEG, aiming at reducing the recognition by immune system, increasing the circulating time in the blood stream, and thus allowing a better control of the in vivo behavior. Moreover, we have developed several approaches (described below) to functionalize the droplet surface by grafting ligands. Our preliminary results regarding the CT scan on mice are summarized in Fig. 2, showing the pharmacokinetics in blood (red curves), liver (open symbols) and spleen (blue curves) of nano-emulsions composed of iodinated vitamin E (left) and iodinated glyceryl monocaprilate (right). Though these nano-emulsions only differ in the composition of the core, their pharmacokinetics is strongly different as one targets the liver, and the other the spleen.

CONCLUSION

Nano-emulsions is a simple system that presents a great potential as contrast agent. In the present abstract, we showed that nano-emulsions are not only very suited for the formulation of CT contrast agents, but also that changing simple parameter like the nature of oil, we can target the imaging properties.

REFERENCES