P-014 **Different iodinated nano-emulsions for preclinical x-ray imaging applications**

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INTRODUCTION AND OBJECTIVES

Microscopic computed tomography (micro-CT) is a high-resolution CT technology which has isotropic voxel with a relatively short scan times for small specimen and small animal researches (Holdsworth 2002). The needs to screen small animals for drug discovery and genomics purposes are quickly increased (Ritman 2002). Because the research on small animal models for human disease is a critical key to understanding the origin, the progression and the treatment of these diseases which cause the major death in the world.

Contrast agent is a substance able to enhance the visibility of structures or fluid within the body. In the case of micro-CT, soft tissues are contrasted using heavy elements like, *e.g.*, iodine. Since the currently used iodinated X-ray contrast present significant limitations, such as their rapid blood clearance, or their high viscosity and the allergic reactions (Bourin 1997, Hallouard 2010), many research efforts have been dedicated to the development of blood pool contrast media able to overcome these problems. Blood pool contrast agents are lipid or polymeric nano-particles containing or encapsulating heavy elements like iodine, or gold for instance. These nano-vectors include liposomes, nano-emulsions, micelles, dendrimers, nanospheres and nanocapsules. To increase their blood circulation times, most of nano-objects have been a surface modified. Another advantage of blood pool contrast agents is their multifunctional properties, both drugs and contrast agent can be introduced into one nano-vector (Hallouard 2010).

Nano-emulsions are formulated by the spontaneous emulsification method (Anton 2008) which takes benefit of the intrinsic physiochemical properties of formulation components. The process of formulation is simple and low-energy emulsification process allowing working with fragile and thermo-sensible molecules. The formulated nano-emulsions can be stable for months at room temperature.

To be a blood pool contrast agent, the iodine is introduced into the oily phase by the Wijs reaction, which saturates the double bonds of fatty acid chains by iodine monochloride. Then this iodinated oil is formulated in the form of nano-emulsions droplets. To increase the iodine percentage in the oily phase and reduce the administrated frequency, a new type of oil is synthesized. It presents a similar structure of Labrafil® compatible with the spontaneous emulsification process, but with higher degree of unsaturation. This study is based on oil in water type nano-emulsions with different iodized oil (iodinated oil of Labrafil M 1944 CS®, Labrafil M 2125 CS® or synthesized oil). The self-emulsified oils are used as oily phase while the aqueous one is a phosphate buffer. A model nonionic surfactant with hydrophilic-lipophilic balance (HLB) of 12-14 was used.

MATERIALS AND METHODS

Materials

Cremophor ELP® (free gift from BASF, France), Labrafil M 1944 CS® (Fagron, France), Labrafil M 2125 CS® (free gift from Gattefossé, France), iodine monochloride (Sigma, USA), linseed oil (Fagron, France), polyethylene glycol 300 (Aldrich, Germany).

Iodinated oil synthesis

The iodinated oil was synthesized following the Wijs reaction. Native oil and iodine monochloride were dispersed in cyclohexane, protected from light and stirred at ambient temperature for 12 hours. The mixture was extracted by ethyl acetate to offer the red iodinated oil. The structure of iodinated Labrafil M 1944 CS® is illustrated in Fig 1.

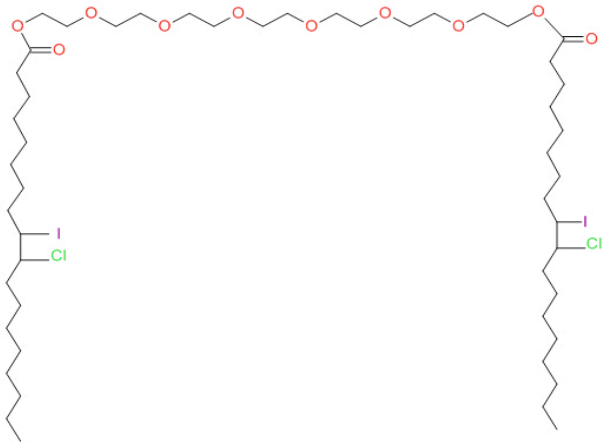

Figure 1: Structure of iodinated oil of Labrafil M 1944 CS®

Oil synthesis

The oil was synthesized according to two main steps. The triglyceride was first hydrolyzed by sodium hydroxide, and then fatty acids obtained were grafted to polyethylene glycol 300 with esterification reaction, and finally purified.

Nano-emulsions formulation

Nano-emulsions were prepared by spontaneous emulsification method. Firstly, the iodinated oil was mixed with non-ionic surfactant (Cremophor ELP®). And then, the mixture of iodinated oil / surfactant was added into the aqueous phase (phosphate buffer) to form nano-emulsions on stirring. The selected formulation was following: SOR = 15% and SOWR = 40%.

(1) surfactant / oil weight ratio: SOR = **W**surfactant / (**W**surfactant +**W**oil)*100; and (2) surfactant-oil/ water weight ratio: SOWR = **W**surfactant+oil / (**W**surfactant+**W**oil+**W**water)*100.

In vivo test of iodinated nano-emulsions

The contrasting power of iodinated nano-emulsions was evaluated by a micro-CT scanner (eXplore speCZT Vision, GE, Waukesha, USA). The selected parameters of micro-CT scanner were following: 70kV, 32mA and 10 msec. The iodinated nano-emulsions of Labrafil M 1944 CS® (0.4ml) were injected by intravenous route into a nude mouse model (37g). The mouse was anesthetized by 1% isoflurance during the test.

RESULTS AND DISCUSSION

In vitro test of iodinated nano-emulsions

The iodine content of the iodinated nano-emulsions was compared with different dilution of XNETIX® 300 (a commercially available hydrophilic contrast media). The result showed that our iodinated product had a significant contrast enhancement compared with Fenestra® which is a commercial *blood pool* contrast product based on nano-emulsions.

In vivo test of iodinated nano-emulsions

After 10 minute of injection in mouse, the contrast enhancement can be seen. The contrast enhancement lasted more than 2h20 after injection. Iodinated nano-emulsions presented a strong accumulation in the bladder which demonstrated the elimination of these iodinated nano-emulsions was performed by the kidney.

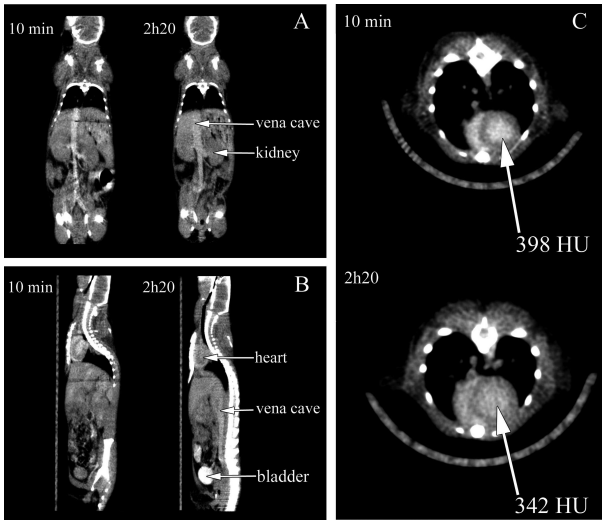

Figure 2: Contrast enhanced images from similar coronal (A), sagittal (B) and axial through the mousse heart (C) slices of the same mousse. Images demonstrate the in vivo contrast 10 min and 2h20 after the intravenous injection of iodinated nano-emulsions with SOR = 15%. The contrast can be always observed in blood pool after 2h20 and the iodinated nano-emulsions are accumulated in the bladder (A and B).

CONCLUSIONS

Three types of iodinated oil with different ratio of unsaturation are synthesized in this study. Iodinated nano-emulsions are formed by spontaneous emulsification method. *In vitro* and *in vivo* experiments demonstrate significantly contrasting properties of these iodinated nano-emulsions. In the future, we are planning to increase the iodinated nano-emulsion residence *in vivo* and hopefully, to increase their tolerance.

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