



In Vivo Behaviour of Cationic Liposome Labeled With In-111 in Mice Using HiSPECT Technique

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INTRODUCTION

HiSPECT is used in this study and it is an extension of single-pinhole SPECT with more than one pinhole in a collimator. Cationic liposomes labeled with In-111 performed with HiSPECT is a good approach in studying the 3D (three dimensional) in vivo biodistribution in mice. This method allows the localisation of liposome at each location in small animals.

LABELLING OF LIPOSOME

200 MBq¹¹¹In-Cl₃ was dissolved in 320µl of NaCl. 175µl of ¹¹¹InCl₃ + 175µl Sodium citrate-Dehydrate Buffer (pH = 4.5). 320µl of the mixture was incubated for 30 minutes with 320µl of cationic liposome solution (DOTAP/DMPE-DTPA (99/1)). Sum of the available amount of labelled liposomes for injection was 634µl with an activity of about 80MBq. Quality control was carried out with a thin layer chromatography which resulted to 98% binding of labelled liposome in the injected solution.

MATERIALS AND METHODS

6 healthy BALB/c male mice (6-8 weeks old) were used for the study and weighed 21.14±1.1g. The mice were anaesthetized by injecting 0.8ml of Ketavet/Rompun and catheter was inserted through the tail veins for the infusion of the radiolabeled liposome. The mouse was positioned under the HiSPECT camera in a special holder.

In-111 labeled cationic liposome of 11MBq was injected. On the first day the dynamic images were acquired for 30 minutes each at time per projection of 47 seconds.

On the second day, MDP used as a clinical bone marker was injected into the mice in order to enable us identify the mice morphology. The mice were measured for 10mins at time per projection of 80 seconds thereby acquiring data. Another static measurement was measured on the third day, for 15 minutes at time per projection of 120 seconds each.

The measured data were transferred and reconstructed using HiSPECT software program for the biodistributions study. ROI analysis were done by placing 40% isocontour regions around the lungs, liver and spleen. The ROI statistics were also evaluated using software program AMIDE.

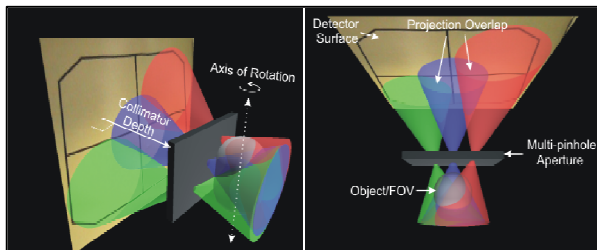
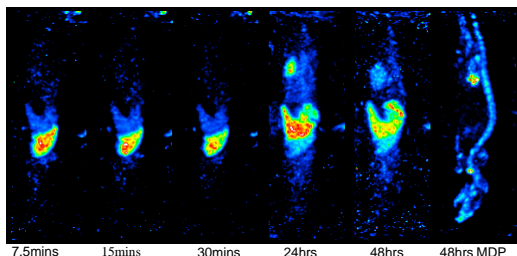


Fig. 1. Principle of multipinhole Technique. The projection overlaps up to 50% without artifacts. High resolution of 0.8mm FWHM and sensitivity up to 2000 cps/MBq was obtained.

Tail



Head

Fig.2. Multipinhole SPECT images of the In-111 labeled liposome uptake behaviour in a male BALB/c mouse after 7.5mins, 15mins, 30mins, 24hrs, and 48hrs. For anatomical orientation we also measured the bone metabolism by co-injection of MDP.

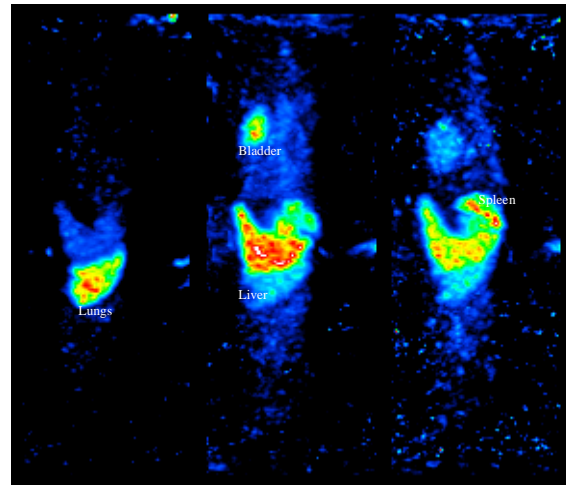


Fig. 3. Liposome uptake after 24hrs p.i in three(3) different mice

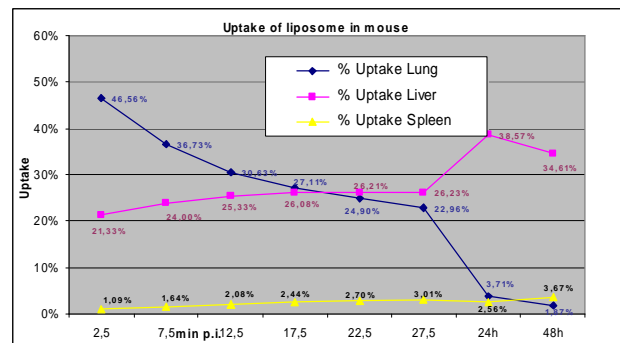


Fig. 4. Graph representation of liposome uptake in different organs against time

RESULTS

A fast uptake and biodistributions of liposome was observed in the lungs of 46.56% which decreased gradually to 1.87%. At the liver there was a gradual uptake of 21.33% liposome, which increases to 38.57% and gradually decreases to 34.61%. At the spleen, the uptake of 1.09% was observed which increases constantly to 3.67% at 48hrs p.i (Fig. 4) The image on the right hand side of Fig. 2 represents the distribution of Tc-99m labelled MDP, which was injected two hours before the 48h measurement of the liposome. Hence, the binding and uptake of cationic liposome occurred very fast in the organs.

DISCUSSION

At the first 30mins, most of the cationic liposome was trapped in the lungs, after 24hrs and 48hrs there was a higher uptake in the liver, spleen and bladder. That means, there was a release of liposome from the lungs into the liver. Hence, we were able to conclude that with the aid of multipinhole SPECT technique, the biodistributions and the fast uptake of the radiolabeled cationic liposomes in the organs of mice can be clearly visualized in vivo without decreasing the image resolution or the sensitivity.