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Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office using the **Electronic Report Submission Application:**

http://193.49.43.2:8080/smis/servlet/UserUtils?start

Reports supporting requests for additional beam time

Reports can now be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

ESRF	**Experiment title:** **Angiogenic targeting of rat brain tumor at Gd K-edge by using cationic liposomes**	**Experiment number**: MD-42
Beamline: ID-17	**Date of experiment**: from: 12.11.2003 to: 17.11.2003	**Date of report**: 01.03.2004
Shifts: 15	**Local contact(s)**: G. Le Duc	*Received at ESRF:*

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Report:

Summary
K-edge imaging experiments in tumor bearing rats with Gd-lablelled cationic liposomes were performed. The aim was to investigate the feasibility of the K-edge imaging technique for determining in vivo the distribution of Munich Biotech's cationic lipid complexes. Measurements with Gd labelled liposomes and with free Gd contrast agent were performed.

Introduction
Cationic liposomes exhibit enhanced binding and uptake at endothelial cells of newly formed (angiogenic) blood vessels [1]. Angiogenesis is involved in a large variety of diseases such as cancer, diabetic retinopathy and chronic inflammation. In the development of solid tumors, formation of new blood vessels is essential for the tumor to grow beyond a size of 1-2 mm. On this basis, Munich Biotech has developed drug loaded cationic lipid complexes which have shown strong anti tumor efficacy [2]. However, the details regarding the correlation between molecular properties of the lipid complexes and selectivity of binding and uptake still need to be elucidated.
K-edge imaging permits to quantify the distribution of a contrast agent in vivo with very good temporal and spatial resolution [3]. This is of particular importance for investigating cationic lipid complexes, because, in contrary to classical liposomes, uptake is thought to occur very fast and at locally confined areas.
For the experiments, tumor (glioma) bearing rats from the medical beamline at the ESRF were selected. Imaging was performed in temporal subtraction mode. Attenuation profiles were measured with a germanium detector. The rats were anaesthetized and a catheter was set. The rats were fixed in a stereotactic frame and positioned vertically in front of the beam on a mechanical motor unit allowing to acquire images in the projection and in tomograpy mode and to determine absolute concentrations of the contrast agent.
Measurements were performed with different types of Gd-loaded cationic liposome formulations and, as a reference, with non-liposomal Gd contrast agent at the same concentration. In those cases about 2 ml of a

solution 30 mM in Gd was applied at about 1 ml/min. For comparison, as well experiments with much higher amounts of free contrast agents were performed (500 mM, 0.5 ml in 1 s).
Most attention was given to the Gd-distribution during and directly after injection. Gd-concentration distribution was determined with temporal resolution of 4 seconds for a period of about four minutes. With the Gd-loaded liposomes, contrast was rather low, but clearly above the detection limit. Continuous contrast increase in the arteries and organs during infusion was determined (Fig 1). The contrast increase in the tumor was somewhat higher that in the surrounding brain. With free Gd contrast agent, Gd-concentration in the arteries was below the detection limit, and the contrast increase in the tumor as well as in the brain was lower.
The control measurement with free contrast agent at higher concentration (Fig. 2) showed, that very fast clearance occurs directly after injection. The concentration decreased exponentially within few seconds. The observation, that with liposomal Gd the concentration remained at a certain level for longer time scales indicates, that the Gd was protected form clearance by the liposomal encapsulation.
Summarizing, the results demonstrate, that K-edge imaging enabled to get insight into the Gd distribution directly after injection and the temporal concentration profiles at a desired site of interest with temporal resolution of few seconds and spatial resolution of hundreds of microns.
For the first time the pharmacokinetics of cationic liposomes was determined in vivo with such high temporal and spatial resolution. Because targeting of cationic liposomes occurs very rapidly, this type of information is of utmost importance for the understanding and the optimization of cationic targeting. The measurements have proven to be principally suitable for screening of formulations for optimum efficacy.
However, in the feasibility studies so far, the contrast was rather low. Therefore, for future studies it is desirable to obtain better contrast. This can be done (i) by higher loading efficacy of the liposomes with the contrast agents, (ii) by using other contrast agents, for instance iodine instead of Gd, or (iii) by using alternative types of cationic carriers, such as solid nanoparticles, which permit to apply much higher amounts of contrast agent.

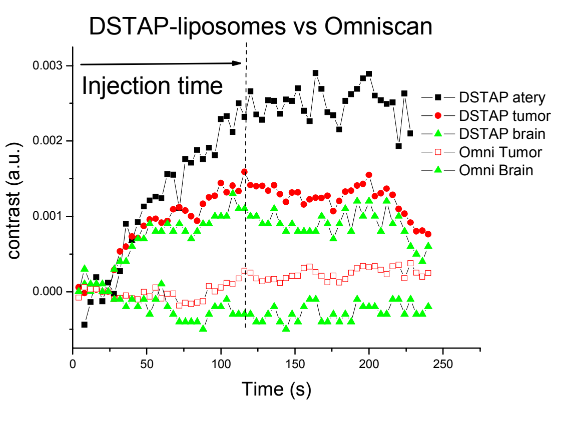

Fig. 1: Contrast during and after injection at different sites for Gd-labelled liposomes and free contrast agent at the same concentration

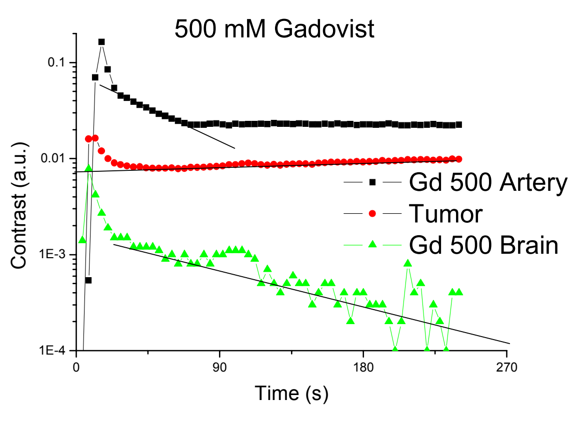

Fig. 2: Contrast after fast injection of Gd contrast agent at high concentration

[1] Thurston G. et al. (1998) J Clin Invest 101, 1401-13
[2] Kunstfeld R et al. (2003) J Invest Dermatol 120(3), 476-482
[3] Elleaume H, Charvet AM, Le Duc G, Esteve F, Bertrand B, Corde S, Farion R, Lefaix JL, Leplat JJ, Berkvens P, Berruyer G, Brochard T, Dabin Y, Draperi A, Fiedler S, Nemoz C, Perez M, Renier M, Suortti P, Thomlinson W, Le Bas JF (2000) Cell Mol Biol 46(6), 1065-75.