Relaxation and diffusion measurements of mobile lipids *in vitro*, *ex vivo* and *in vivo*

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NMR-visible mobile lipids have been detected in cultured cells, and *in vivo* and *ex vivo* in animal and human tumours. They have been found important for tumour diagnosis and therapy monitoring, especially brain tumours. NMR-visible mobile lipids originate from the mobile fatty acid chains, mainly from triacylglycerols with some contribution of cholesteryl esters. Their localisation has been debated for a long time. They were thought to arise from lipid microdomains (22-28 nm in diameter) embedded in the membrane bilayer or from lipid droplets located within tumour cells (around 1 µm in diameter) or from lipid droplets present in the extracellular space, within necrosis (> 1 µm).

Relaxation time and diffusion measurements were performed in several studies to characterize the rotational and translational mobility of the NMR-visible mobile lipids, and as a consequence to bring some understanding on their organisation and location. These different studies will be presented and discussed during this morning-categorical course.

While the T_1 values reported in the literature for the methylene peak of the NMR-visible mobile lipids (1.3 ppm) were all within the same range (between 300 and 500 ms), the T2 values were much more heterogeneous. The first T_2 measurements reported very long T_2 values (around 800 ms) in rat mammary adenocarcinoma cell suspensions (1). Shorter T_2 values (between 120 and 220 ms) were found for glioma cells in suspension or *ex vivo* in gliomas undergoing apoptosis (2). Even shorter T_2 values (between 30 and 60 ms) were measured *in vivo* in gliomas, brain metastases and in lymphomas (3,4). This heterogeneity will be discussed with regards to the multiexponential relaxation behaviour of the methylene peak at 1.3 ppm, due to the overlap with lactate and also with regards to a possible effect of droplet size or to the presence of lipid droplet-associated macromolecules.

Diffusion ^{1}H MRS studies were first performed at one diffusion time *in vivo* in rat gliomas. The root mean square displacement provided a rough estimate of the minimum size of the lipid compartment, around 2.6 and 3 µm in C6 glioma (5) and BT4C glioma undergoing apoptosis (6,2). Further experiments performed with varying diffusion times demonstrated the restriction of the NMR-visible mobile lipids *in vivo* in C6 gliomas (7) and in C6 cells (8). A mean characteristic compartment diameter could be estimated respectively around 4.3 and 1.9 µm, in line with the presence of lipid droplets up to 10 µm in diameter *in vivo* in C6 gliomas and with the mean characteristic diameter of the cytoplasmic droplets estimated from microscopy in C6 cells (around 1.4 µm). These diffusion studies indicate that mobile lipid signal detected by ^{1}H MRS in gliomas arises mainly from cytoplasmic lipid droplets in glioma cell cultures (8), from cytoplasmic droplets in hypoxic or apoptotic cells and in necrosis *in vivo* (7).

References

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