CANCER: Brain and Other: Animal Studies

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$^1$H NMR spectroscopy (MRS) observes truly non-invasively a biologically distinct class of cellular lipids *in vivo*. It is commonly accepted that the MRS-detected lipids reside in lipid vesicles (or lipid bodies) [1], that are over 0.1 μm in diameter [2]. These $^1$H MRS detectable lipids are known by several names in the literature, such as 'MR-visible lipids' and 'mobile lipids (ML)'. It is commonly accepted that $^1$H MR spectrum of non-proliferating cells outside adipose tissue, muscle, myocardium and thymus contains no MLs [1]. Ground-breaking observations by Mountford and co-workers that ML patterns of lymphoblastoid cells differ as a function of cell transformation or differentiation [3] and that malignant transformation of lymphocytes results in strong increase in MLs [4] attracted substantial research efforts to characterize cancerous cells and tissues *ex vivo* by high-resolution MRS [4].

*Assignment of $^1$H MRS lipid peaks in vivo*. Use of $^1$H MRS *in vivo* for detection of MLs is not unproblematic due to narrow chemical shift range of proton nucleus and resulting overlap of chemical species. Established localized data acquisition techniques, i.e. STEAM and PRESS, are commonly used for *in vivo* $^1$H MRS. While these techniques provide good spatial accuracy, spectral overlap in the aliphatic chemical shift region hampers unambiguous assignment of NMR peaks. Peak centered at 1.3ppm, commonly called as 'a lipid peak', gains contributions not only from lactate $–CH_3$ and lipid $–CH_2$-, but also from proteins and polypeptides [5] as well as from free amino acids [6]. Similarly, the peak around 2.0 ppm can have contributions from N-acetylaspartate (NAA), NAA-glutamate (NAAG), glutamate and lipid $=CH-C\mathbf{H}_2-CH_2$. Special NMR techniques, such as (a) editing techniques exploiting J-coupling, e.g. band-selective inversion with gradient dephasing (BASING) [7] or echo time filtering, (b) diffusion filtering [8] (c) coherence selection according to multiple quantum transitions [9] or (d) 2-dimensional MRS [10] should be used for unambiguous assignments of these chemicals. While assignment, and consequently quantification, of saturated lipids requires care due to spectral overlap, peaks from unsaturated lipids, such as those from $=CH-C\mathbf{H}_2-CH=$ at 2.8 ppm and $–C\mathbf{H}=C\mathbf{H}-$ at 5.4ppm, suffer much less from co-resonating species [11].

*Animal models* for common pathologies, such as cancer, neurodegeneration and neuroregeneration, have been extensively studied by means of $^1$H MRS with a view of searching for (a) common metabolite patterns for diagnosis in given condition as well as (b) potential metabolite changes in response to successful treatment outcome.
*Models for cancer*: MLs by $^1$H MRS have been detected in model tumours originating from several cell lines *in vivo*, including gliomas [12,13], neuroblastoma [14] and lymphoma [8]. Saturated lipid signals are commonly detected in localized $^1$H MR spectra from highly malignant tumour xenocrafts.

Anti-cancer drug treatment of neuroblastoma xenocrafts has been shown to result in extensive increase in the ratio of 1.3ppm peak-to-choline containing compounds (Cho) in responding tumours, but not in drug resistant tumours [14]. Both saturated and polyunsaturated $^{1}$H MRS lipids accumulate in BT4C gliomas during cytotoxic gene therapy [12,15] and in EL-4 lymphomas during etoposide treatment [8]. It has been proposed that $^{1}$H MRS detected saturates and polyunsaturates may serve as treatment biomarkers for responsive tumours [1].

Diffusion $^{1}$H MRS has indicated that ADC of saturated and unsaturated lipids does not change during tumour cell kill through apoptosis, unlike that of Cho that decreases [12]. Unchanged lipid ADC during cell kill is consistent with idea of $^{1}$H MRS lipid signal arising from intracellular lipid vesicles [16,17]. Recent analysis of $^{1}$H MRS and water ADC showed that increase in MRS-quantified –C**H**=C**H**- and cholesterol compounds was associated with water ADC increase in the early phase of apoptotic cell death in a rat glioma [18]. Biochemical and molecular biology evidence from a rat glioma undergoing apoptotic cell death points to activation of phospholipase A2 as the molecular mechanism underpinning for intracellular lipid repartitioning that renders them $^{1}$H MRS detectable [19].

*In vivo* MRS has been complemented with *ex vivo* NMR analysis of tissue specimens using high-resolution magic angle spinning (HR-MAS) MRS. HR-MAS provides excellent spectral resolution approaching that obtained in solid state NMR. HR-MAS has confirmed increase in both saturated and unsaturated lipid [20], both MRS lipids showing strong inverse relationship with cell count during cytotoxic gene therapy [21]. Unlike MRS-detected lipids, total Cho signal, as quantified by HR-MAS, remains unchanged in dying BT4C gliomas with cell count decreasing by up to 70% [22].

*Neurodegeneration due to stoke:* MR techniques are particularly suited for both diagnosis of acute ischaemia and monitoring of progression of post-ischaemic processes, including level of oedema, inflammation, neurodegeneration, and formation of gliosis [23]. It is well appreciated that in the core of irreversible ischaemic lesion $^{1}$H MRS detected metabolites strongly decrease during progression of infarction [24,25]. Following a short ischemic exposure much less subtle $^{1}$H MRS alterations have been reported, including increase in MRS lipid signals [26]. Histology shows delayed lipid accumulation under these conditions [27]. It has been suggested that the $^{1}$H MRS detectable lipids reside in activated microglial and/or exogenous macrophages [26], a hypothesis that has been recently questioned [28]. Instead, the proposal is that lipids become MRS-visible due to ongoing neurodenegeration *per se* [28].

*Neuroregeneration.* Adult brain retains ability to generate new neurons in a limited fashion owing to presence of neural stem and progenitor cells, found in hippocampus and subventricular zone [29]. Recently, a study was published indicating that neural progenitor cells (NPC) in culture express lipid species with a $^{1}$H MRS resonance at 1.28 ppm that is found neither in cultured neurons nor glial cells [30]. This putative $^{1}$H MRS 'biomarker' for NPC was assigned to a mixture of saturated and monounsaturated fatty acids. Using standard PRESS for localized $^{1}$H MRS and a post-processing algorithm based on singular value decomposition, it was claimed that spectra from hippocampus of both rat and

human, but not that of cerebral cortex, contain the putative 'NPC biomarker' peak at 1.28 ppm [30]. These *in vivo* MRS assignments have been recently questioned [31-33]. A recent study using $^{1}$H MRS found no such 1.28 ppm peak in either murine embryonic stem cells or NSC, but rather reported varying levels of choline-containing metabolites in these stem cell cultures [34]. Interestingly, high concentrations of MLs have been during myoblast fusion in muscle indicating that regeneration process may render cellular lipids MRS visible in muscle tissue [35].

*Lipid data provided by $^{13}$C and $^{31}$P MRS:* Barany and co-workers [36] assigned several natural abundant $^{13}$C peaks in the rat brain *ex vivo* originating from fatty acyl side chains of membrane phospholipids. Due to low sensitivity and need for incorporating exogenous labeled substrates, $^{13}$C MRS has not gained use for lipid work *in vivo*. This situation may chance in near future owing to sensitivity enhancement by dynamic nuclear polarization of substrates for metabolic studies *in vivo*.
$^{31}$P MRS provides large chemical shift difference for biochemicals linked to membrane phospholipids metabolism, such as phosphocholine, glycerophosphocholine, phosphorylethanolamine and glycerolphosphoethanolamine. Owing to these qualities $^{31}$P MRS is extensively used to study these metabolites in cancer models [37], and it is also gaining clinical applications [38].

In conclusion, $^{1}$H MRS detectable lipids show 'biomarker' value in several forms of cancer and neuro-pathologies. Preclinical work is paving an avenue for several potential clinical applications for the $^{1}$H MRS ML resonances.

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