Hyperammonia and hypoxia induce relevant changes in lipid signals in ^{1}H NMR spectra from human cancer cells

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INTRODUCTION

The technique of NMR has provided new insights on cell metabolism in animals and humans, due to the capacity of studying biologically active molecules in intact cell systems. With this respect, the intense lipid signals attributed to fatty acid chains in neutral lipids have elicited much attention in the NMR community, aimed at providing new tools to improve the knowledge in lipid metabolism and correlate the spectral observations to biologically relevant events. We therefore studied the effects of hyperammonia and hypoxia conditions in tumour cells in culture in order to provide more insight on these lipid metabolites and possible changes in their synthesis.

METHODS

All cells were grown as adherent cells as described elsewhere [1]. Cells were treated with 20 mM NH_4Cl for 24 hours before NMR experiments. Hypoxia was obtained by growing cells for 24 hours in 2% O_2, 93% N_2, 5 % CO_2. ^{1}H NMR spectra were run at 400.14 MHz on a digital Avance spectrometer (Bruker, AG, Darmstadt, Germany) equipped with a 1mm microprobe. Signals were acquired with a 90° RF pulse and a sweep width of 4006.4 Hz. Water suppression was obtained by irradiating water signal. Lorentzian-Gaussian resolution enhancement was used.

RESULTS AND DISCUSSION

The region of high lipid resonances in a typical spectrum of HeLa cells is shown in Fig.1a. Fatty acid bulk methylene protons resonate at 1.28 ppm (Lip), very close to the lactic acid methyl group at 1.32 ppm (Lac). Terminal methyl group and methylene protons from fatty acid chain show signals both in 1D and 2D COSY spectra and can be used for area quantitation. According to current models, these signals are from neutral lipids, mostly triglycerides.

Cells were then treated with NH_4Cl. HeLa cells displayed significant increase of Lip signal (Fig.1b). The same results were observed in 2D COSY spectra as well as in two not-related cell lines, MCF-7 from breast cancer and T98G from glioma (not shown). Similar effects were observed by other authors in F98 glioma cells, by means of ^{13}C NMR, pointing to increased synthesis of fatty acids and di/triglyceride, though after shorter time intervals [2].

The same effects on Lip signals were obtained when cells were kept under low oxygen conditions, as shown in Fig 2 a and b for HeLa cells, where a relevant increase in Lip signal is present in hypoxic cells (Fig. 2b). In the past, hypoxic state has been associated to triglyceride and free fatty acid accumulation [3]. As it is expected that hypoxia may alter intracellular pH, this finding is also in agreement with a possible effect of decreased intracellular pH, that was shown to increase lipid signals in C6 glioma cells [4].

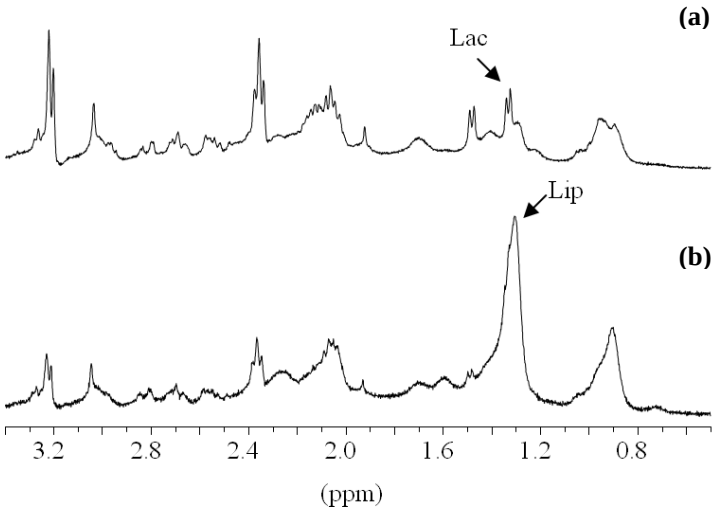

Figure 1: *High field region of ^{1}H NMR spectra of HeLa cells. Upper trace: control sample; lower trace: NH_4Cl treated cells. Spectra were run after a 24 h treatment with 20 mM NH_4Cl.*

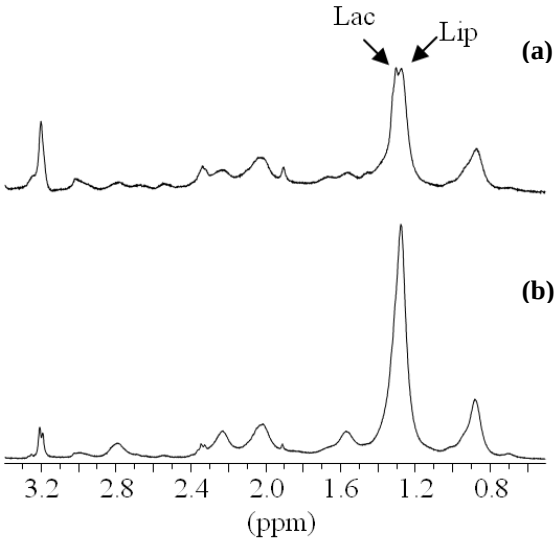

Figure 2: *High field region of ^{1}H NMR spectra of HeLa cells. Upper trace: normoxic cells. Lower trace: hypoxic cells.*

CONCLUSIONS

Tumour tissues are often characterized by intense signals from fatty acids that have been widely studied to provide possible markers of tumour metabolism. Present data show that ^{1}H NMR can be used to detect accumulation of neutral lipids due to conditions, such as hyperammonia or hypoxia, that are frequently encountered in tumour tissues, as well as in other pathologic situations, pointing to a possible use as detectors of cell response to modified environment.

REFERENCES

[1] Rosi, A. et al., Radiat. Res. 167, 268-282 (2007).

[2] Zwingmann C. and Leibfritz D. in: *Advances in Hepatic Encephalopathy and Metabolism in Liver Disease*, Record C and Al-Mardini H eds., Newcastle (2000).

[3] Gordon G.B. et al., American Journal of Pathology 88: 663-674 (1977)

[4] Barba et al., Cancer Research 59(8): 1861-1868 (1999).