Origin of the Mobile Lipids detected intensity changes in C6 glioma cells with proliferation rate

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Introduction

NMR-visible mobile lipids (ML) signals resonating at 1.28ppm and 0.9ppm have been described in the spectral pattern of aggressive tumours (glioblastoma and metastasis) and in various cultured cell types[i]. These ML mostly originate from triacylglicerol (TAG) in droplets (1-10 micrometers of diameter)[ii iii] and have been related to necrosis and hypoxia in tumours [iv] and proliferation rate in cultured cells[ii]. Proper understanding of the biochemical and biophysical origin of these ML could help MRS of human brain tumours to provide useful information for diagnosis, prognosis and therapy planning [v]. C6 cells display variable ML content (increase by 5-27 times) with proliferation arrest under defined culture conditions [ii, vi].

Purpose

To ascertain whether ML intensity changes with proliferation rate in normally growing C6 cells are caused by biochemical changes (variation in TAG content per cell) or a different origin needs to be considered.

Methods

C6 total lipid cell extracts [vii] of cultured cells (day 4 cells, log phase, n=3, and day 7 cells, postconfluent, n=3) and {1}-^{13}C-glucose 99% enriched grown cells (day 4 cells, n=3, 24h incubation with ^{13}C enriched glucose, day 3-day 4, and day 7 cells,n=3, 48h incubation with ^{13}C enriched glucose, day 5-day 7) were studied by ^{1}H-^{13}C HMQC (TD=2K, NS=64, Ninc=256), in an ARX Bruker 9.4T (SeRMN, UAB) and processed using Xwinnmr software (Bruker, Germany). Quantitation of neutral lipid content in C6 lipid extracts was performed by TLC on silica-glass plates that were Coomassie-blue stained and densitometrically evaluated [viii]. Data analysis (non-parametric test for 2 independent variables) was performed using SPSS 11.5 for Windows (SPSS Inc., USA), with significance threshold set at $p<0.05$.

Results and discussion

Quantitation of C6 lipid extracts by TLC and ^{1}H-^{13}C HMQC showed no statistically significant changes in TAG content between day 4 and day 7 cultured cells (Tables 1 and 2). In ^{1}H-^{13}C HMQC experiments of extracts of C6 cells incubated with {1}-^{13}C-glucose, the ratio TAG α', γ/choline (normalized as in Table 2) increased 4.3 times between day 4 cells and day 4 (3+1) labeled cells, and 11.3 times between day 7 cells and day 7 (5+2) labeled cells. On the other hand we found an increase of 50 times in the glycerol α carbon of phospholipids (PLα) /choline ratio between day 4 and day 4-labeled cells, while this ratio increased 26 fold between day 7 and day 7- labeled cells. Synthesis of PL and TAG is necessary for cell duplication if their content is to remain constant. Then, PL content per cell does not change between log and post-confluent cells (result not shown), and TAG content per cell, (Table 1 and 2), does not change between the two phases. On the other hand, PL labelling, during log phase incubation with {1}-^{13}C-glucose, is about 10 times higher than TAG labeling for the same time interval. These labeling suggests a differential cellular compartimentalization of the synthesis pathway for PL and TAG, additionally requiring an origin different from glucose for TAG synthesis under the experimental conditions (log phase and post-confluence) here assayed.

	μgrams /10^8 cells	μgrams /mg prot
4 days cells (n=3)	86.30 ± 23.38	4.79 ± 1.3
7 days cells (n=3)	110.18 ± 39.37	6.88 ± 2.5

Table1 TAG content quantified by TLC.

assignment	Crosspeak volume/choline* (mean value x 10^{-2} ± sd)	
	4 day cells (n=3)	7 day cells (n=3)
TAG α', γ	3.71±0.27	3.76 ± 0.33

Table 2 ^{1}H-^{13}C HMQC experiments of C6 lipid extracts (*) Ratio of TAG cross-peak volume normalized to phosphatidilcholine (cross-peak volume at 3.27-54.85ppm).

Conclusions

In normally growing C6 cells in culture, ML detected intensity changes related to proliferation rate may be caused by changes in the biophysical properties (e.g. NMR visibility) of the ML but are not caused by changes in the TAG content. Net TAG synthesis required to maintain TAG content per cell constant does not originate directly from glucose, like for PL in the experimental conditions here assayed, but from a different source.

References

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