^{1}H-MRS mobile lipid localization in rat brain glioma

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Introduction

Localization of mobile lipid detected *in vivo* by ^{1}H-MRS has been widely discussed during the past years. Lipid signal is supposed to arise either from plasma membrane (1), from intracytoplasmic droplets (2), or large compartments (a few µm diameter) in association with necrosis (3). In addition, presence of lipid droplets in growing number and size from the periphery toward the necrotic center of the tumor have been observed (3). These datas suggest that lipid droplets are a good candidate for the location of mobile lipids detected by ^{1}H-NMR; and detection of this lipid signal might be linked to necrotic process. So, according to this hypothesis, ^{1}H-MRS lipid signal should only be detected by the end of tumoral growth. Smaller lipid droplets have been observed in the cytoplasm of viable tumor cells at the periphery of necrosis. According to literature (4), presence of lipid droplets in these cells is a good indicator of stress originating from hypoxia (4). If this hypothesis is right (5), staining of hypoxic cells and of lipid droplets should be found colocalized. Experiments were done to prove or not these hypothesis.

Methods

First we have analyzed by histology, the kinetics of appearance of necrosis and lipid droplets during tumoral growth. This study was performed on 34 female Wistar rats implanted with C6 cells. The rats were divided into 7 groups and sacrificed 7, 14, 18, 21, 24, 27 and 30 days after cell implantation. The brains were excised and frozen. Sections (10 µm thick) were stained with HE and 2 specific lipid stainings (Red Oil and Nile Red). Secondly, presence of ^{1}H-MRS mobile lipids has been correlated with necrosis and lipid droplets, using an other group of animals (8 rats). NMR experiments were carried out 15, 18, 21, 25 and 28 days after C6 cell implantation. 1D spectra (TE=136 ms) and 2D J-resolved maps were acquired (PRESS sequence) from the tumor and the contralateral tissue. The rats were sacrificed as soon as a NMR lipid signal was detected and histology was performed. Finally, hypoxia and lipid droplets stainings were assessed on 5 rats, 22 to 25 days after C6 cell implantation. A hypoxia marker (Pimonidazole) and a perfused vessel marker (Hoescht) were injected intravenously respectively 1h and 1 min before the rat death. The brains were removed and freezed. Immunohistology was performed on the sections (5 µm thick).

Results

Neither necrosis nor lipid droplets were observed before 2 weeks postimplantion. Small necrotic area and low amount of lipid droplets appeared 18 days after cell implantation. These results were more significant from 21 days postimplantation: large areas of necrosis surrounded by cells arranged in pseudo-palisade characterized the tumors. Lipid droplets were observed essentially inside necrosis and in lower amount and smaller size in a cell layer around necrosis. Mobile lipid signal was detected from 21 days postimplantation (Fig.1 and 2). For all the rats, detection of a NMR lipid signal can be associated with presence of large areas of necrosis containing lipid droplets (Tab.1). Furthermore, intensity of lipid resonance seems to be well correlated with necrosis expansion and amount of lipid droplets. Hypoxic cells were detected all around necrosis. However, only the hypoxic cells nearby necrosis contained lipid droplets.

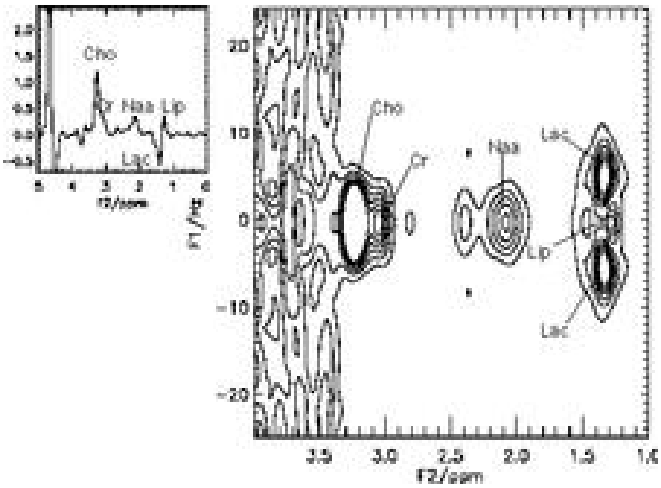

Fig. 1: 1D spectrum and 2D J-resolved map

Discussion

Our results clearly demonstrate the narrow relationship between lipid droplets and ^{1}H MRS lipid signal detected *in vivo*; Our conclusion is in accordance with recent results of the literature (6, 7). The correlation between ^{1}H MRS mobile lipids and necrosis has also been shown, confirming Kuesel et al. results (8) on human brain tumor biopsies. Necrosis is surrounded by hypoxic areas, but as lipid droplets were only observed within hypoxic cells very close to necrosis, we hypothesized the existence of a hypoxic gradient from the periphery towards the necrotic center of the tumor. In conclusion, mobile lipids detected *in vivo* by ^{1}H MRS in a model of C6 rat glioma, arise from lipid droplets mainly found in necrosis. Thus, the detection of a high intensity of 1H-MRS lipid signal could constitute a signature for a very necrotic tissue and indicate the tumor grade. Presence of lipid droplets in viable tumor cells can be considered as an indicator of a prenecrotic hypoxia.

References

1. C.E. Mountford et al., Trends in Biochemical Sciences 13, 172-177 (1988)
2. R. Callies et al., Magnetic Resonance in Medicine 29, 546-550 (1993)
3. C. Rémy et al., Cancer Research 57, 407-414 (1997)
4. I. Freitas et al., Anticancer Research 10, 613-622 (1990)
5. J.A. Raleigh et al., Radiation Research 151, 580-589 (1999)
6. I. Barba et al., Cancer Research 59, 1861-1868 (1999)
7. J. Hakumaki et al., Nature in Medicine 5, 1323-1327 (1999)
8. A.C. Kuesel et al., NMR in Biomedicine 7, 149-155 (1994)

Tab.1: Correlation between NMR lipid signal, lipid droplets and necrosis

	NMR lipid signal	Lipid droplets	Necrosis
Q02 (21d)	+	+	+
Q09 (21d)	+	+	+
P99 (21d)	+	+	+
Q01 (25d)	-	-	-
Q03 (25d)	+	+	+
Q00 (25d)	+	+	+
Q04 (28d)	+	+	+
P97 (28d)	+	+	+