Metabolic profile of lipid extracts obtained by astrocytic brain tumors

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Introduction

Astrocytic brain tumors – Gliomas in particular – are the most common brain tumors in adults. These malignant neoplasms are characterized by heterogeneous tissue with no distinct differentiation to healthy tissue because of their infiltrative growth. Therefore, the ratio of tumor cells versus healthy tissue in the tumor margin needs to be determined very precisely when the decision is made to which extent this tissue has to be resected surgically.
As high resolution magnetic resonance spectra of intact tissue or tissue extracts reflect numerous metabolic information, the metabolic composition of tissue samples from the tumor margin and the tumor core were recorded. In addition, analysis of ^{1}H-NMR spectra with pattern recognition algorithms permits a tumor classification, since different neoplastic tissue types show different metabolic profiles. Therefore, 22 astrocytic brain tumors were analyzed with ^{1}H-NMR spectroscopy, subdivided in 22 biopsates of tumor core, 14 biopsates of tumor margin and 4 reference extracts of normal tissue.

Methods

Tissue samples were extracted using a dual phase extraction technique with chloroform, methanol and bidistilled water. The organic extracts were dried under nitrogen, weighed and redissolved in 0.6 ml deuterated chloroform-methanol (2:1). The lipophilic components were analyzed with a Bruker Avance DRX-600 at 300K using a 5 mm H,C,N inverse probe with actively shielded gradient coils. Signal assignments of ^{1}H-NMR spectra were confirmed using heteronuclear 2D-NMR spectra.
Eight lipid extracts of tumor core tissue were separated using solid phase extraction (SPE) with aminopropyl columns using a slightly modified method after Kaluzny et al.[1]. The obtained SPE fractions were dried under nitrogen and re-dissolved after evaporation in 0.6 ml deuterated chloroform-methanol (2:1). 1D ^{1}H-NMR spectra of the SPE fractions were recorded as well as various homo- and heteronuclear 2D NMR spectra for structure identification.

Results

Various lipids were observed within the lipophilic tissue extracts. Beside different fatty acids, neutral lipids like cholesterol (C), cholesterol ester (CE) and to a minor content tri- and diacylglycerides (TAG, DAG) were detected. Among polar lipids glycerophosphocholines and –ethanolamines occurred as well as plasmalogens, sphingomyelins (SPH) and galactosyl cerebrosides (GS). The latter represent a metabolic discriminator: GS were present in extracts of reference tissue in particular with a ratio of GS to SPH ≈ 3.33± 0.22 (fig. 1). However, GS were not or to a minor content observed in 20 spectra of the tumor core, with GS/SPH ≤0.25± 0.004. Extracts of the tumor margin show distinctly variable GS contents i. e. no GS was detected in two extracts, a minor amount of GS was observed in five extracts while seven extracts showed GS contents like in the reference extracts (average GS/SPH ≈ 1.25± 0.16). This reflects the distinct variable of tumor to normal cells.
Furthermore, ^{1}H-^{13}C-HSQC spectra revealed olefinic protons in spectra of tumor core tissue, which appear upfield shifted compared to signals of unsaturated fatty acids (fig. 2). These resonances were detected in 20 of 22 extracts of tumor cores, but they were absent in all reference spectra and in 9 of 14 spectra of the tumor margin. Therefore, eight extracts of tumor core tissue were separated by SPE into the different lipid classes (fig. 3) in order to facilitate structure elucidation. The unknown olefinic signal was preferentially observed within the CE and TAG fractions. These fractions were combined and various homo- and heteronuclear 2D spectra were recorded. Using the structure information obtained from different 2D spectra and chemical shifts of lipid standards analyzed for comparisons, the olefinic signal could be identified as an isoprene derivative (fig. 4). Due to the small amount of this derivative and overlapping signals especially in the high field part of the spectra a definite assignment of the remaining residue R is not possible so far.

Conclusion

Analysis of 22 astrocytic brain tumors showed different amounts of galactosyl cerebrosides in the distinct tissue types and may serve as a tissue discriminator. In addition, analysis revealed an unknown olefinic compound in tumor core tissue. It was not observed in spectra of healthy tissue and it was detected in only a few spectra of the tumor margin. This compound shows an unpolar character and is assigned to an isoprene derivative.

References:

[1] M.A. Kaluzny et al., *Journal of Lipid Research* 1985; Vol. 26; 135-140.

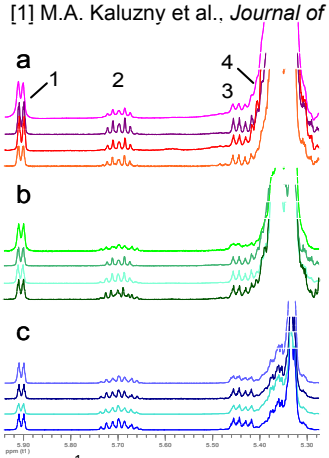

Fig. 1: ^{1}H-NMR spectra of a) tumor cores b) tumor margins and c) reference tissue. Double bond resonances of plasmalogens (1), sphingomyelins and galactosyl cerebrosides respectively (2,3) and fatty acids (4,cutted off) are shown.

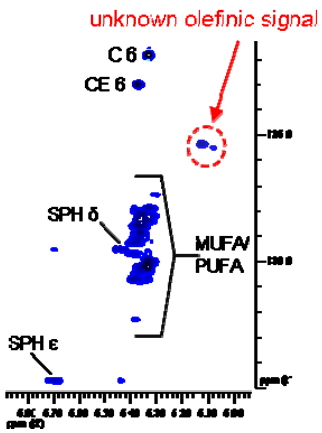

Fig. 2: Olefinic section of a ^{1}H^{13}C-HSQC spectrum of a tumor core tissue extract.

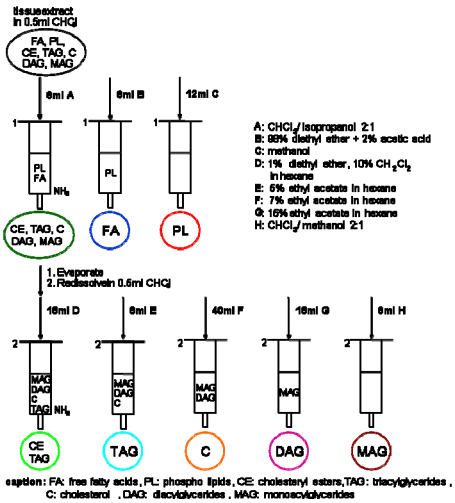

Fig. 3: Elution pattern for lipid separation.

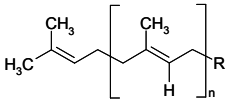

Fig. 4: Assumed sub-structure of the olefinic compound.