Investigation of postmortal decomposition processes in pig brain by in situ and high-resolution 1H-NMR spectroscopy

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Purpose:

The time of death is an important information in forensic medicine for the reconnaissance of suspicious deaths. Usual indicators for estimation of the post mortem interval (PMI) like methods mainly based on body temperature, are limited to the first 2 days after death. However, chemical degradation and decomposition processes can be observed over substantial longer periods of time. In this study post mortem changes in the chemical composition of pig brain tissue was investigated during the first 3 weeks by in situ and high-resolution ^{1}H-NMR-spectroscopy. The brain was chosen due to its protection against environmental influences and the small interindividual differences in its chemical composition.

Methods and Materials:

Ten heads of young pigs (age: 8-9 weeks, weight: ca. 30 kg) were serially investigated for up to 3 weeks in a clinical whole body scanner (1.5 T). ^{1}H-MRS measurements were performed with single volume selection using a PRESS sequence (TR/TE = 1500ms/135ms) and a conventional transmit/receive quadrature head coil. The voxel position was controlled by T_1-weighted images. Measurements started with a typical voxel size of 6 ml. During the time course of the experiments the voxel size was further reduced down to 1 ml to avoid contamination by liquor, bone or developing gas bubbles. Depending upon the availability of the scanner measurements were repeated in time intervals of 8 to 24 h. In some cases measurements had to be terminated prematurely due to field inhomogeneities caused by strong bubble formation. In 5 cases perchloric acid (PCA) extracts of the brain were prepared at different PMIs (1.1; 4.2; 8.7; 13.6; 21.2 days, see Fig. 1). These extracts were investigated by high-resolution 1D-^{1}H-NMR (600 MHz; 14.1 T) and by 2D-DQ-COSY-^{1}H-NMR (750 MHz; 17.6 T).

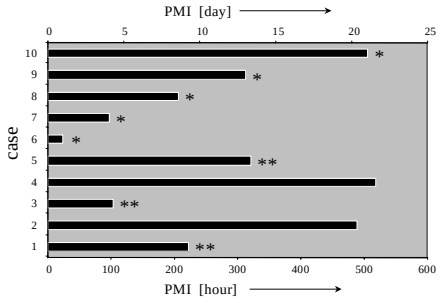

***Fig. 1**: Time periods of in situ ^{1}H-MRS for the 10 pig heads. Cases with preparation of PCA extracts are indicated by (*). Two asterisks (**) indicate premature termination.*

Results:

The in situ spectra indicated a continuous degradation of N-Acetylaspartate (NAA 2,0 ppm). Creatine (Cr 3,0 ppm), choline (Cho 3,2 ppm) and lactate (Lac 1,3 ppm) remained approximately constant during the first days but decreased rather quickly later. After 3-4 days new compounds, like acetate (Ace 1,95 ppm), succinate (Suc 2,4 ppm) and free trimethylammonium (fTMA 2,9 ppm), appeared in the spectra and increased strongly. These increases were accompanied by gas formation as seen in the MR-images. The compounds were identified by high-resolution 1D-NMR (Fig. 2a). Additional compounds, such as amino and carboxylic acids, were identified by high-resolution 2D-NMR spectra (Fig. 2b). A comparison of all investigated brain spectra revealed similar time courses of alteration for the different metabolites. The signals were assigned according to the data published in [1] and [2].

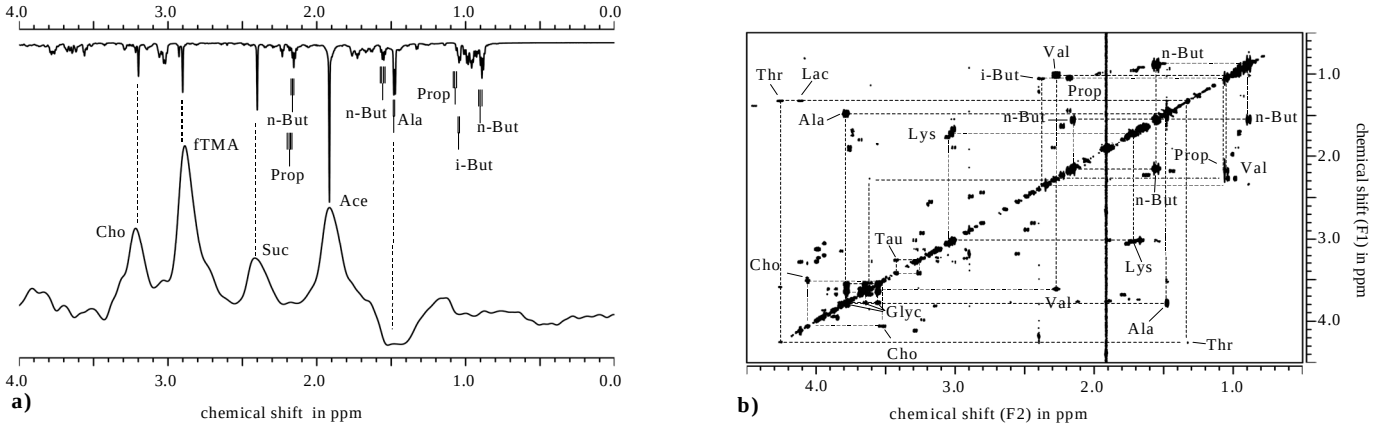

Fig.2: (a) Comparison of in situ MR spectrum (1.5 T) of a pig brain at 8.7 days after death (lower spectrum, left) and the high-resolution NMR spectrum of the corresponding PCA extract at 14.1 T (upper spectrum, left). (b) Besides the metabolites indicated in (a), further carboxylic and amino acids were detected in the 2D-DQ-COSY spectra at 17.6 T (right spectrum). Only the most intensive signals of n-butyric acid (n-But), iso-butyric acid (i-But), propionic acid (Prop), alanine, valine (Val), threonine (Thr), Lysine (Lys) as well as glycerine (Glyc), choline (Cho) and lactate (Lac) are assigned.

Conclusions

^{1}H-MRS spectra reflect post mortem decomposition processes due to autolysis (reduced NAA) and putrifaction (increasing Ace, Suc, fTMA, gas formation). The similarity of the progressing metabolic changes observed in all samples indicates that autolytic and bacterial decomposition processes in the brain may be useful for PMI determinations. In addition to the evaluated uncoupled resonances of the ^{1}H-MR spectra substantially more metabolites were identified by the 2D NMR techniques. Their relevance as potential markers has to be assessed in further studies.

References

[1] Govindaraju et al.NMR Biomed. 2000 May;13(3):129-53. [2] Ith M *et al.* Magn Reson Med. 2002 Nov;48(5):915-20.