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SHORT COMMUNICATION

Comparison of post-mortem metabolic changes in sheep brain tissue in isolated heads and whole animals using ^{1}H-MR spectroscopy—preliminary results

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Abstract By means of in situ proton magnetic resonance spectroscopy (^{1}H-MRS) time-dependent metabolic changes in brain are measured for the estimation of post-mortem intervals (PMIs). An isolated whole head and a whole young sheep from an abattoir with known time of death were stored at approximately 21°C, and localized ^{1}H-MRS (TR/TE 2,500/25 ms) of the brain was performed at various PMIs with a clinical 1.5 T whole-body scanner using a quadratur head coil. In the isolated head, additional metabolites including free trimethylammonium (fTMA), propionate, and butyrate, and especially acetate, could be detected as described in previous studies. In the head of the whole animal, especially fTMAs and lactate were found in the late PMI but no further metabolites of relevance. These preliminary findings support the hypothesis that in a whole-body corpse, the distribution of bacteria from the gastrointestinal tract up to the brain in the late post-mortem interval can influence the metabolic decomposition of brain tissues. In further studies, it should be kept in mind that an isolated head does not represent authentic circumstances and is, therefore, not an ideal model for brain decomposition in intact corpses.

Keywords Proton magnetic resonance spectroscopy · Post-mortem metabolic changes · Sheep · Post-mortem interval (PMI)

Introduction

A reliable estimation of the post-mortem interval (PMI) is an obvious task in criminal investigations, but the value of chemical methods for this purpose is up to now very limited [1, 2]. Especially when putrefactive changes are apparent, only a rough estimation of the PMI is possible based on subjective experience of the forensic pathologist, but not as a scientifically sound method.

About 30 years ago, extensive experimental work was carried out on the protein degradation during putrefaction in various organs, e.g., the brain by Bonte [3–5] and later by Daldrup [6–8], who proposed a method of death time calculation on the basis of amino acid concentrations in the brain. However, all these methods never gained real practical relevance.

A few years ago, first results concerning post-mortem decomposition of brain tissue have been described by using proton magnetic resonance spectroscopy (^{1}H-MRS) in a sheep head model and selected human cases [9]. At first, changes in the concentrations of metabolites usually present in vivo and the appearance of previously reported decay products were observed. About 3 days post-mortem, new metabolites including free trimethylammonium (fTMA), propionate (Prop), butyrate (But), and iso-butyrate started to appear in situ. Observed metabolites and time course were comparable in sheep and human brain tissues. These findings could be confirmed using isolated whole heads of young pigs, where the time course of spectral changes was observed within 3 weeks [10]. In situ ^{1}H-MRS was

Dedicated to Prof. Dr. Thomas Daldrup on the occasion of his 60th birthday.

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considered as a possible useful tool for the estimation of PMIs. Scheurer et al. [11] proposed mathematical functions to describe the time course of characteristic metabolites in decomposing brain (sheep head model) up to 3 weeks post-mortem. Individual PMIs calculated gave a good correlation up to 250 h post-mortem. Recently, also a Chinese group proposed a mathematical model for the estimation of PMIs using ^{1}H-MRS at different temperatures [12].

However, previous groups used isolated heads of animals, which were prepared by closing the spinal canal with plasticine, fixing in a plastic holder and storing in a plastic container or bag. The aim of the present preliminary study was a comparison of the metabolic changes in an isolated whole head and a head of an intact animal.

Material and methods

Experimental setting

An isolated whole head of a young sheep with known time of death was obtained from an abattoir. Immediately after decapitation, the spinal canal was sealed by plasticine in order to prevent loss of cerebrospinal fluid and to avoid direct bacterial contamination of the brain. Afterwards, the head was placed in a plastic container and stored at room temperature (21±3°C) for the entire period of investigation of up to 14 days post-mortem. Temporarily shifted whole young sheep obtained from an abattoir was stored in a plastic container under the same conditions. ^{1}H-MRS was performed at several points of time post-mortem. Unfortunately, the number and planning of measurements were regulated by clinical routine diagnostics in the hospital, so the possibilities were rather limited. The spectroscopic volume of interest was positioned in the parieto-occipital region of the sheep brain, yielding a mix of gray, and white matter brain tissue.

Instrumentation and method

Single-voxel ^{1}H-MRS was performed with a clinical 1.5 T whole-body MR scanner (Philips Healthcare, Best, The Netherlands) using a conventional transmit/receive quadrature head coil. Image-guided localized spectra were acquired by using a PRESS sequence (point resolved spectroscopy) with water suppression (TR/TE=2,500/25 ms, voxel size=5 ml).

Results and discussion

Gas bubbles occurring in the brain tissue after 6 to 7 days in the isolated head and 4 to 5 days in the head of the whole animal complicated the selection of a voxel entirely within homogeneous brain tissue and impaired spectroscopic measurements at longer PMIs.

During short PMIs, spectra obtained from the isolated head (Fig. 1) showed signals already described by others and also occurring in healthy living brain, like the singlets of *N*-acetyl-aspartate (NAA) at 2.01 ppm (total) creatine (tCr) at 3.02 ppm, and a single peak of bound trimethylammonium, mainly including choline compounds at 3.19 ppm [13]. After a short time, the CH_3-doublets of lactate (Lac) at 1.33 ppm and alanine (Ala) at 1.47 ppm appeared. Nine hours post-mortem acetate (Ac) was detected only as a small upfield shoulder of the NAA peak. At longer PMIs, Ac became the predominant peak and additional signals evolved for succinate at 2.41 ppm and fTMA at 2.88 ppm, whereas other metabolites like NAA, tCr, and Lac disappeared.

In the present study proton MR spectra congruent with those from previous studies [9–11] were observed. This

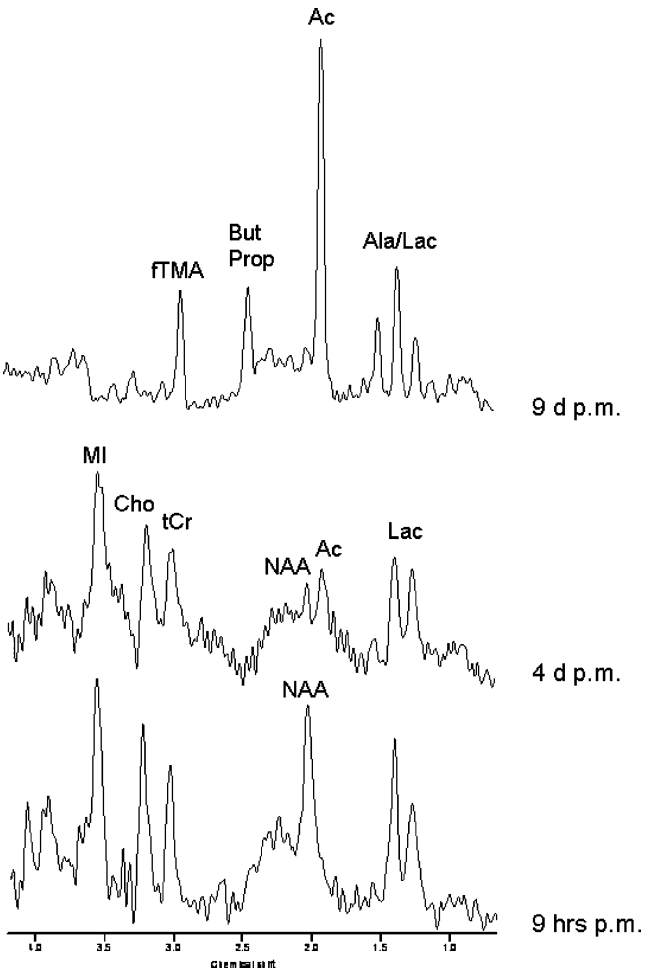

Fig. 1 Metabolic decomposition in an isolated brain of a sheep measured by ^{1}H-MRS on a 1.5 T MR-system (TR/TE 2,500/25 ms) 9 h, 4 days, and 9 days post-mortem. The data of other groups were highly reproducible (*MI* myo-inositol, *Ac* acetate, *Ala* alanine, *fTMA* free trimethylamine, *But* butyrate, *Prop* propionate)

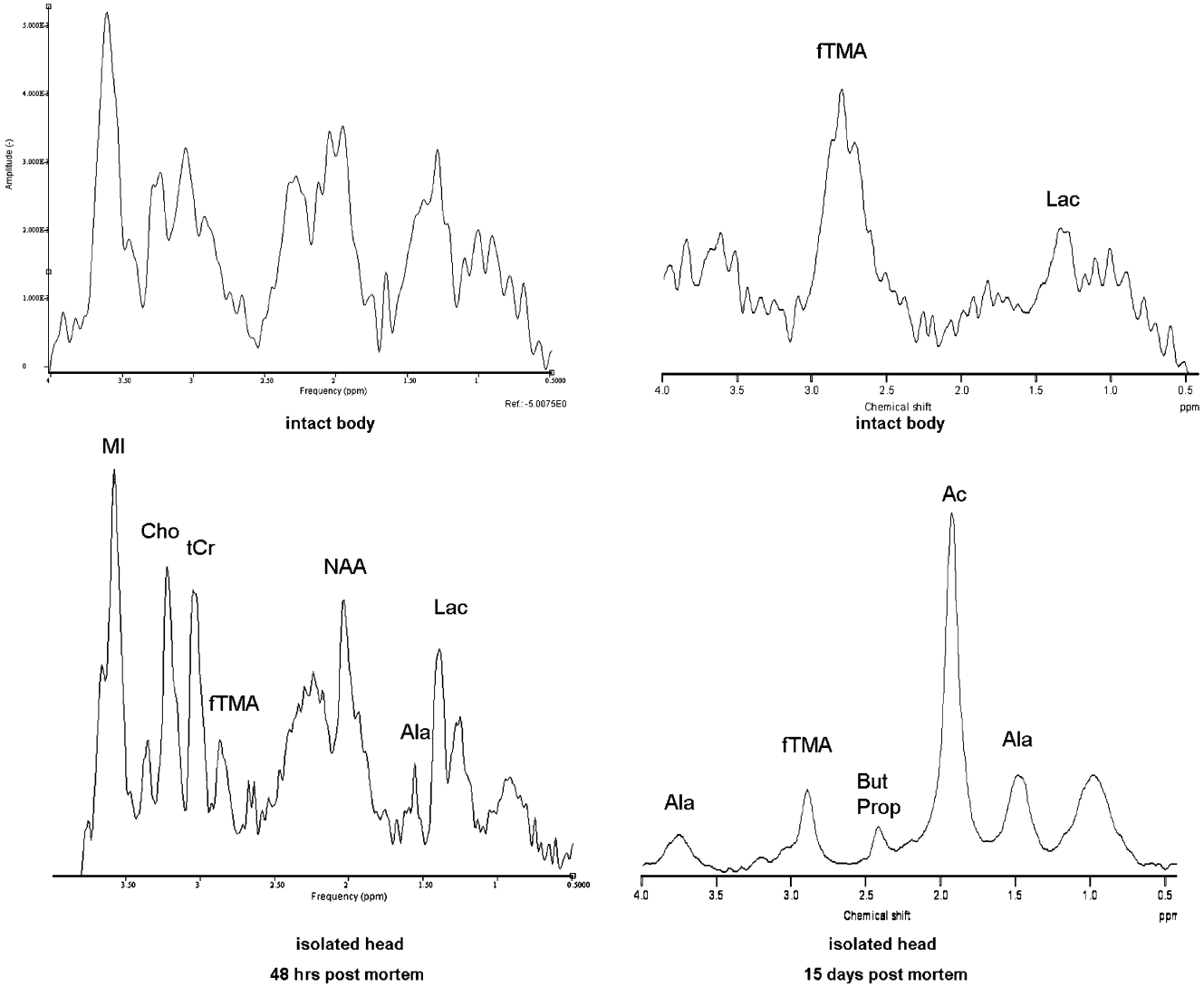

Fig. 2 ^{1}H-MRS of sheep brain analyzing an intact body and an isolated head 48 h and 15 days post-mortem (*MI* myo-inositol, *Cho* choline, *tCr* total creatine, *NAA* *N*-acetyl aspartate, *Ac* acetate, *Lac* lactate, *Ala* alanine, *fTMA* free trimethylamine, *But* butyrate, *Prop* propionate)

indicates that the animal model is highly reproducible and in principle can be seen as a useful tool for further studies.

But quite different results were revealed by investigation of the metabolic alterations in the brain of the whole animal. Spectra showed differences already 48 h post-mortem, but especially after longer PMIs (Fig. 2). Fifteen days post-mortem mainly fTMAs and Lac were found in the intact animal, but no further metabolites like Ac, Prop, Ala, and But, which were used for a mathematical model for the estimation of PMIs [11]. These were only detectable in the isolated head 15 days post-mortem.

Indeed in a previous publication the statistical model based on animal data with isolated heads was applied to four human cases and showed a good correlation [11]. However, the bodies were only measured at one point in time and were stored at 4°C for between 20 and 70 h before MR examination.

Due to the limited number of cases and measurements the present findings can only be seen as preliminary results. However, significant differences in metabolic alterations during post-mortem decomposition in brain were demonstrated between the animal model with isolated heads and the intact animal. Decomposition in the intact animal appeared faster and probably different compared to the model with isolated heads, due to bacterial invasion from the gastrointestinal tract, which can reach the brain in a few days in a cadaver. The significance of putrefiers like *Clostridium sordelli* for the estimation of the PMI was demonstrated by Bonte [3] or Daldrup and Huckenbeck [7] several years before.

However, the investigation of post-mortem decomposition by ^{1}H-MRS may represent a real progress in research. The procedure allows a non-invasive chemical analysis in situ with quantitation of analytes as well as longitudinal

studies of post-mortem changes with reproducible results. Influencing factors like temperature and temperature-dependent invasion ability of bacteria can be easily studied, but in further studies intact bodies should be used instead of isolated heads. Old questions in forensic medicine like death time estimation can benefit from recent developments in modern imaging systems.

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