1H-MRS and metabolomics for diagnostic activity assessment of hydatid cysts of the parasite *Echinococcus granulosus*

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Introduction

Hydatid disease is caused by cysts (metacestode) of the larvae of the parasite *Echinococcus granulosus* (canine band worm) and is a significant public health problem in South America and in mediterranean and middle eastern countries, for example. Man serves as an intermediate host, and the liver is the most common site of infestation. The cysts have the form of multilaminar, fluid-filled capsules with an inner germinative membrane which generates brood capsules containing protoscolesces, the active infectious agent for the final canine host. Common diagnostic imaging methods such as ultrasound, CT, and MRI do not readily allow one to make the important distinction between "fertile" (metabolically active) and "sterile" (inactive) cysts – valuable information for therapy planning and follow-up. A small number of ^{1}H MRS studies, both in vitro [1-3] and in vivo [4,5], have indicated that metabolite profiles for the cyst fluid may prove clinically useful for fertility assessment or for distinguishing hydatid cysts from other types of cysts [6]. A common result of these studies was the detection of high levels of lactate, acetate, alanine and TCA cycle metabolites in active cysts. However, a major metabolite exhibiting a singlet resonance at 2.41 ppm has been variously assigned as pyruvate [4,5] or succinate [1-3,6].

The purpose of our ongoing study is to use high-field ^{1}H-MRS to obtain detailed metabolite profiles from *E. granulosus* cyst fluid obtained from patients and to correlate these profiles with classical diagnostic parameters and staging criteria.

Materials and Methods

In our pilot study the current data set covers 16 samples of cyst fluid (obtained by aspiration or interventional surgery) from 13 patients (5 male, 8 female, mean age 39 years). Cysts were classified sonographically according to WHO staging criteria (2001). The fluid samples exhibited a wide range of physical characteristics: from a clear, low-viscosity fluid to an opague gel-like consistency with tissue fragments. The samples were examined microscopically to judge protoscolesces activity (integrity, mobility).

Samples of fluid (ca. 0.5 ml) were placed in 5-mm NMR tubes and mixed with 30 µL of D_2O containing the reference compound trimethylsilyl propionic acid (TSP). Sample pH was ca. 7.8. ^{1}H NMR spectra were acquired at 600 MHz (14.1 T) without sample rotation using a Bruker BioSpin AV-600 spectrometer. The water signal was suppressed by presaturation. In addition to conventional 1D spectra, a number of 2D NMR experiments were performed (COSY, NOESY, J-resolved, and in a few cases TOCSY and inverse ^{1}H/^{13}C correlation) to assist in the assignment of metabolite signals. Assignments were made on the basis of chemical shifts and homonuclear coupling patterns by comparison with literature data. Relative concentrations for 22 metabolites were calculated from the ^{1}H signal integrals in the 1D spectrum and normalized to valine. Due to the heterogeneity of the samples with high viscosity, the determination of absolute metabolite concentrations was not attempted. Broad signals for proteins, unsaturated lipids, and *N*-acetyl groups of saccharides were also detected and evaluated in a semiquantitative manner as "very low, low, moderate, or high".

Results

On the basis of clinical examination, a total of 10 cysts were classified as "active" (stages CE1-CE3a), three were termed "intermediate" (CE3a, CE3b) and three were considered to be "inactive" (CE4, CE5). The signal linewidths increased with sample viscosity and lipid content, independent of protein content. Lipid content did not correlate with classification or staging, but protein content and *N*-acetyl signal intensity tended to be very low for active cysts and moderate to high for intermediate and inactive cysts. Metabolites that could be identified and quantitated included several amino acids (alanine, valine, leucine, isoleucine, glycine, histidine, tyrosine, phenylalanine), lactate, acetate, formate, succinate (confirmed via H/C shifts of 2.41/36.91 ppm), citrate, malate, fumarate, glucose, *myo*-inositol, betaine, choline, and others. Pyruvate was notably absent in all samples. The metabolite profiles provided no discrimination between the so-called "intermediate" and "inactive" cysts, and these two subgroups were taken together for subsequent group comparisons. Furthermore, the data for one "inactive" sample were discarded because evaluation of the very broad spectrum (gel-like sample with bubbles) was uncertain and incomplete. Table 1 summarizes those metabolite ratios which exhibited a significant difference between the active and inactive classes. Various amino acids, citrate, glucose, and choline metabolites provided no discrimination between the two classes.

Conclusions

In this pilot study with a small number of *Echinococcus* cyst fluid samples, a number of metabolite/valine ratios show promise for fertility assessment. The ^{1}H MR spectra of active (fertile) cyst fluid are characterized by high levels of lactate, succinate (not pyruvate!), acetate and malate, as well as detectable fumarate, indicating high activity of the TCA cycle. While lactate ratios increase further in inactive (sterile) cysts, the ratios for the other diagnostic metabolites in Table 1 decrease dramatically. The results also indicate that the ratios acetate/lactate and succinate/lactate have high diagnostic potential and should be measurable noninvasively with in vivo ^{1}H-MRS in a clinical setting.

Table 1. Group comparisons for metabolite/valine ratios in cyst fluid (*t*-test, two-sided, unequal variance)

Class (*n*)		Lactate	Succinate	Acetate	Malate	Betaine	Fumarate
"active" (10)	**mean**	16.1	6.05	2.20	1.17	0.85	0.19
	SD	13.3	3.37	1.28	0.87	0.63	0.12
"inactive" (5)	**mean**	34.9	0.28	0.34	0.0	0.16	0.004
	SD	8.0	0.17	0.12	0.0	0.08	0.008
	***p* value**	0.0052	0.0004	0.0012	0.0021	0.0072	0.0011

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