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PROTON NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY OF PLASMA LIPOPROTEINS IN MALIGNANCY

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Abstract

A recent study (N. Eng. J. Med. 315 (1986), 1369), described a method of detecting malignant tumors by water-suppressed proton nuclear magnetic resonance (1 H NMR) study of plasma. We performed a similar study of the W 1/2, a mean of the full width at half height of the resonances of the methyl and methylene groups of the lipids of plasma lipoproteins which is inversely related to the spin-spin apparent relaxation time (T 2*). W 1/2 values were measured at a fixed baseline width of 310 Hz. The study was prospective and blinded and comprised 182 subjects consisting of 40 controls, 68 patients with untreated malignancies, 45 with malignant tumors undergoing therapy and 29 benign tumor patients. No differences were seen between any groups that could serve as a basis for a useful clinical test. The major difficulty in the determination of W 1/2 was due to interference of metabolite protons (particularly lactate) within the lipoprotein resonance signal. Triglyceride level was seen to correlate inversely with W 1/2 within malignant patient groups. These discrepant results may be related to differing triglyceride-rich very low density lipoprotein (VLDL) levels in the patient populations of each study. We conclude that the water-suppressed 1H NMR of plasma lipoproteins is not a valid measurement for assessing malignancy.

Key words: Plasma lipoproteins, NMR spectroscopy, cancer detection.

The availability of a test to detect and follow human malignancy has long been an aim of clinical medicine (8). While certain tumor markers are useful in this regard, they mainly cover a few tumor types (1, 5), and the large majority of cancers are not thus characterized. Recently there has been excitement over detection of changes in patient plasma analysis by water-suppressed proton nuclear magnetic resonance that, purportedly, is able to detect malignant conditions (4).

In order to confirm this approach, we undertook the study of a large population of patients with malignant and benign conditions, and are now reporting this analysis.

Material and Methods

The total study material included 182 subjects: 40 controls (C), 68 patients with untreated malignant tumors (UM), 45 patients with progressive malignant tumors under therapy (TM) and 29 patients with benign tumors (BT) seen at the Centre Georges François Leclerc, Dijon, France. The composition of the patient population with malignant tumors is shown in Table 1.

A prospective blinded study was performed employing a technique similar to that previously described (4). A 400 MHz Brucker WM spectrometer was utilized, and the proton nuclear magnetic resonance of plasma studied after water suppression by presaturation at the frequency of water resonance. The proposed parameter (W 1/2) is defined according to the following equation: $W\,1/2 = 1/2\,[\mu 1/2)1 + (\mu 1/2)2]$ where $(\mu 1/2)1$ is the full width at half height of the methylene peak and $(\mu 1/2)2$ the corresponding measure for the methyl peak. Thus W 1/2 is a mean of the full width at half height of the resonances of the methyl and methylene groups of the lipids of plasma lipoproteins which is inversely related to the spin-spin apparent relaxation time (T 2*). This latter parameter was measured at a fixed baseline width of 310 Hz and was expressed in Hertz (Hz) (Fig. 1).

Triglyceride (TG) and total cholesterol (TC) levels were determined by an enzymatic method. Serum apolipoprotein B containing lipoproteins were selectively precipitated by concanavalin A (3). After centrifugation, HDL-

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Table 1

Patient population with malignant tumors

Type of malignancy	Untreated malignant tumors (UM)	Malignant tumors under treatment (TM)
Breast	24	17
G.I. tract	12	3
Prostate	11	4
Head and neck	2	7
Lymphoma	3	6
Other uro-genital	2	4
Lung	3	2
Gynecologic	3	2
Unknown primary	5	–
Other	3	–
Total	68	45

cholesterol (HDL-C) was measured in the supernatant by an enzymatic method.

Means of W 1/2 and TG parameters in C and UM-TM-BT patient groups were compared with Student's t-test after having employed the F-test for equality of variance.

Results

Table 2 shows the distribution of mean values of W 1/2 and of the lipid parameters in the 4 patient groups. The W 1/2 mean value showed a difference between the C and TM groups ($p=0.04$). The means for the C, UM, and BT groups were quite similar. No separation point could be identified between any groups on scatter diagrams (Fig. 2).

The triglyceride levels correlated inversely ($r=-0.62$, $p<0.01$) with the W 1/2 values within the malignant groups (UM, TM) (Fig. 3). No correlation was found between W 1/2 and total cholesterol, HDL-cholesterol and (LDL+VLDL)-cholesterol levels.

Discussion

In our analysis of 182 patients, we failed to confirm the results of Fossel et al. (4). Thus, we did not find the water-suppressed NMR analysis of plasma-lipoprotein to be a valid test for the detection of an underlying malignancy, or in fact to even correlate with an obvious malignancy. The scatter diagrams for the different groups were fairly similar and did not constitute a basis for a useful diagnostic test.

Several points need to be discussed concerning the discrepency between our results and those of Fossel et al. (4).

Firstly, we chose a fixed baseline width of 310 Hz (Fig. 1), while a specific line width was not described in the study of Fossel et al. A difference in baseline width would be expected to yield a difference in W 1/2 (7).

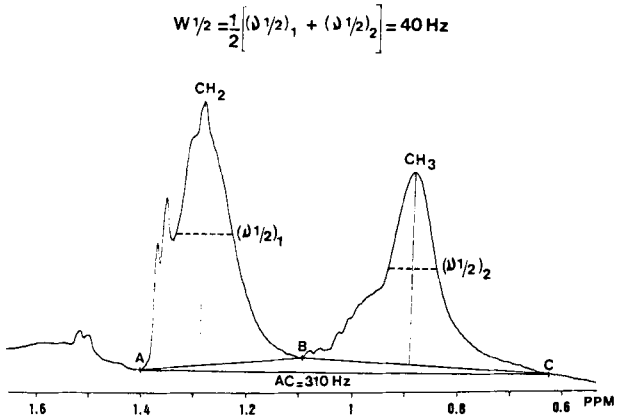

Fig. 1. 400-MHz water-suppressed proton NMR spectrum of methyl and methylene region of control plasma. Line widths were determined by drawing a fixed line and then measuring the heights of the resonance. The half-heights are determined and the full widths are measured and converted into Hertz by multiplying them by the scale of the plot in Hertz per cm.

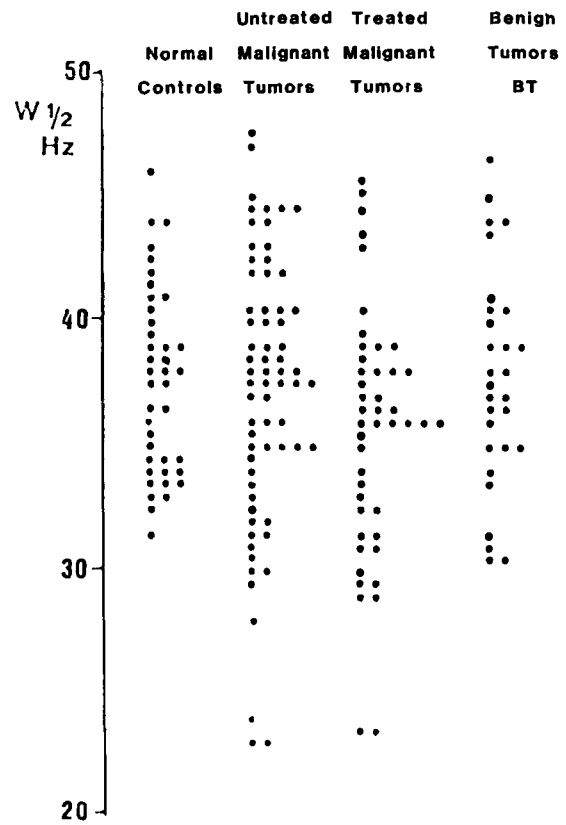

Fig. 2. Distribution of W 1/2 parameter in the control and patient groups.

Secondly, the presence of certain metabolic by-products interferes with the lipoprotein NMR signal and introduces uncontrolled parameters in the W 1/2 measurement (Fig. 4). One of these by-products is lactate, known to be frequently elevated in patients with malignancy (2,

Fig. 3. Distribution of W 1/2 parameter in malignant patients (UM, TM) according to triglyceride levels (TG).

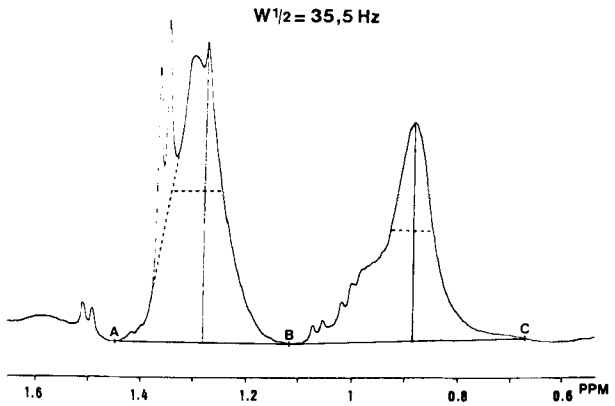

Fig. 4. 400-MHz water-suppressed proton NMR spectrum of methyl and methylene region of patient plasma with the presence of metabolic by-products interfering with the lipoprotein NMR signal.

Table 2

Age, lipid parameters and results of W 1/2 in the four patient groups (mean±SD)

	Controls	Malignant tumors, under treatment	Malignant tumors, untreated	Benign tumours
n	40	45	68	29
Age (year)	33.0±6.9	60.9±11.8	63.4±13.1	71.6±15.7
TC (mg/l)	1 855±273	2 032±448	2 102±607	1 814±349
HDL-C (mg/l)	641±117	489±195	526±183	500±153
(LDL+VLDL)-C (mg/l)	1 215±261	1 543±418	1 574±588	1 314±352
TG (mg/l)	625±316	1 289±607	1 242±1186	874±334
		p=0.0001*	p=0.001*	p<0.01*
W 1/2 (Hz)	37.6±3.7	35.6±5.0	37.1±5.5	37.7±4.3
		p=0.04*	37.1±5.5	37.7±4.3

TC = Total cholesterol, HDL-C = HDL cholesterol, (LDL+VLDL)-C = (LDL+VLDL)-chlolesterol, TG = triglyceride.

* p compared to control group.

6). Since neither the study of Fossel et al. nor our study measured lactate levels, this could represent a confounding factor in both studies.

Thirdly, we found an inverse correlation between triglyceride levels and W 1/2 within the malignant groups, another confounding variable not specifically measured by Fossel et al. (4). Our results suggest that the decrease in NMR line width seen in some patients with malignant tumors may be related to an increase to triglyceride-rich very low density lipoproteins (VLDL).

In the study of Fossel et al., it was noted that no benign conditions (other than pregnancy and benign prostatic nodules) had W 1/2 below 33 Hz. In our study all normal controls had W 1/2 levels above 31 Hz, but this was the case also for most patients with benign or malignant tumors (Fig. 2).

Although we have found that water-suppressed NMR plasma lipoprotein analysis, as performed, is not a useful test for detection of malignancy, it would nevertheless be interesting to perform an analysis on lipoprotein subfractions. Small changes that might be evident by such an analysis may be hidden by shifts in the lipoprotein subfraction distribution not detected by the current overall method. We are in the process of such an analysis.

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