혈장 지단백질에 대한 핵자기 공명 분광 분석

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The proton nuclear magnetic resonance spectral analysis of human blood plasma lipoprotein

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Abstract

300 MHz proton NMR spectra of human blood plasma were analyzed by deconvolution of spectrum, and we compared its results with Fossel's test in normal (15 cases), liver cancer (14 cases), and other cancer (14 cases) groups. This analysis had enabled us to obtain dynamic characteristics of each individual lipoprotein.

As a result of deconvolution method, the VLDL and chylomicron intensity level were found to be elevated in the patients with liver cancer. Moment ratio values of CH_2 resonance in the raw spectrum were found to be higher than the normal group for patients with malignant tumors other than liver cancer. These differences between the three groups could not be found in the conventional Fossel's test.

We could simulate plasma spectra by addition of spectra of individual lipoproteins through deconvolution method. Further clinical trials in larger populations and additional biochemical method may shed new light on many of clinical and biochemical interests for knowing charateristics about lipoprotein not separated from blood and the background of Fossel test

INTRODUCTION

Since Fossel et al. first began the study on the detection of malignant tumors using the proton NMR spectrum in plasma a number of laboratories had undertaken duplication of the results and modifications of the NMR technique.

Most of these laboratories reported substantial overlap among patient's groups, which clouds the diagnostic potential of the Fossel test. Neither Fossel nor others had a satisfactory explanation on the breaking point characteristics or overlap between normal group and cancer groups.

A simulation method of the proton NMR spectra for lipids has been used to get information on the dynamic properties of methyl and methylene groups of membrane phospholipid. This study may provide a basis on the question on Fossel test using the proper dynamical model of the aliphatic group.

Jimmy et al. reported that the methyl and methylene resonances of plasma lipoproteins have composite peaks representing a number of

nearly equivalent but unresolved components. In this case Fossel test may be a chemical shift phenomenon.

On the basis of this report we were able to obtain information on the distribution function of lipoproteins in plasma and the line shape using the deconvolution method such as curve fitting and maximum likelihood estimation

MATERIALS AND METHODS

Plasma samples

Blood was collected in vacutainer tubes containing EDTA. Samples of blood were drawn after a 16 hr. fast in all 43 subjects. The bloods were kept at 4 °C before centrifugation. Plasma was obtained after centrifugation (rpm: 3000) for 10 min at 4 °C. Plasma was kept at 4 °C before NMR measurements.

Patient groups

Group 1 consisted of 15 healthy hospital personnel as normal controls. Group 2 comprised 14 patients with liver cancer. Group 3 comprised 14 patients with malignant tumors other than liver cancer.

Healthy hospital personnel served as normal controls. Two patients' groups were selected after a careful review of each patient's medical records, particularly the clinical and pathological diagnosis in the study.

NMR spectroscopy

All proton NMR spectra were obtained without knowledge of the patients' clinical status,at room temperature(23 to 24 °C), at 300 MHz, with use of Bruker A.M. 300 spectrometer.

All samples (300μl) were prepared in tubes with an outside diameter of 5 mm(Wilmad Co.).

The large H O signal was suppressed by binary pulse sequence D_2O was used in external procedure. Each spectrum corresponds to 16 free induction decays. The spectrum which includes only the methyl group is shown in Fig.1.

NMR spectrum analysis

1) The mean line widths of methyl and methylene resonances were measured using the same method Fossel used.

2) The proton NMR spectra of human blood plasma consist of a broad envelope of overlapping resonances from macromolecules. The most prominent resonances near 0.8 and 1.3 ppm are assignable to lipid methyl and methylene groups

The methyl and methylene resonances of HDL (CH_3 :0.858 ppm) and LDL(CH_2 :1.25 ppm,CH_3 :0.863 ppm) are shifted slightly to lower frequency and broadened compared to those of chylomicron(CH_2 :1.285 ppm,CH_3 :0.894 ppm) and VLDL(CH_2 :1.279 ppm,CH_3 :0.886 ppm). These experimental results are shown in Fig.12.

We were able to discriminate the overlapping features using the curve fitting method. From NMR theory the line shapes of NMR spectrum were classified into two classes; Gaussian type and Lorentzian type.

In this study we assumed the line shapes of spectrum for each lipoprotein were gaussian type. Then the total simulated spectrum can be described as follows.

$$F(w) = \Sigma Fi(w) \quad i=1,..,6. \quad \text{---------(1)}$$

$$= \Sigma Fi(w) \quad i=1,..,4. \quad \text{---------(2)}$$

$$Fi(w) = Ai*\exp(-((w-wi)^2/\sigma i)/2)$$

w : frequency

wi : chemical shift(unit:Hz) of each lipoprotein resonance (Chylomicron and VLDL, HDL and LDL),Lactate1 and Lactate2

σi : standard deviation of each lipoprotein resonance(unit:Hz)

Ai : relative peak value of each lipoprotein resonance

* The differences in frequency for each lipoprotein relative to chylomicron and VLDL(wi) are as follows in equation (2).

w = -8.8 Hz for HDL and LDL

w = 12.0 Hz for Lactate1

w = 19.0 Hz for Lactate2

In equation (2), the approximation is possible as the assignment in resonance for VLDL is nearly equivalent to that for chylomicron; also the assignment for HDL is equivalent to that for LDL.

The calculated parameters were the standard deviation and relative intensity using the least squares method The standard deviation is related with T2, spin-spin relaxation time,which shows the degree of molecular motion. That it's value is small means the motion is fast Also the simulated spectra were used to obtain the moment ratio which was used for determination of the line shape.

The individual curve-fitted spectra were shown in Fig.2, and the reconstructed spectrum was in Fig.3.

From the simulated spectrum, we caculated the intensity and moment ratio

Intensity $= \int_{-\infty}^{\infty} F(w)\, dw$ where F(w) is line shape and w is frequency

Moment ratio = $M_4 / (M_2)^2$ where Mn is defined as follows

$$Mn = \int_{-\infty}^{\infty} w^n x\, F(w)\, dw \Big/ \int_{-\infty}^{\infty} F(w)\, dw,$$

n = 1,2,..

* We analyzed the only CH2 group in(2).

* Moment ratio is the same concept used as kurtosis statistically.For gaussian shape,the moment ratio value is 3.

Statistical analysis

Kruskal-Wallis analysis was employed to test the null hypothesis in comparing groups. All P values are estimated in the one-sided test.Kruskal-Wallis statistic is defined as H.

RESULTS

Error percentages(%) between the raw spectrum (Fig.1). and the simulated spectrum (Fig.3) were calculated as 13 ±4 % for group 1,13 ±5 % for group 2, and 12 ±4 % for group 3.

The results are summarized as follows.

1) Fossel test

The mean line width in methyl and methylene resonances was measured; 32.9 ±7.3 Hz for group 1,30.3 ±5.7 Hz for group 2,and 23.6 ±8.4 Hz for group 3. There was no correlation between group 1 and malignant tumors(Fig.11). There was no difference of the line widths among three groups (P > 0.02, H=8).

2) Moment ratio

Moment ratio values caculated from reconstructed curve-fitted spectra(Fig.7) are overlapped for the normal group(2.87 ±0.19) and the patients' groups (3.08 ±0.18 for group 2,3.03 ±0.22 for group 3) similar to the results of those of raw spectra(Fig.8), but the mean values for the patients are higher than those for the normal group. There was no correlation between moment ratio value and malignant tumors.

There was no difference in the moment ratio values amonng three groups(P > 0.02, H=7.53)

3) Intensity

The intensity defined in this study is not a real concentration of lipoproteins but a integral value which was measured from the NMR spectrum. As a result of curve fitting, the elevated chylomicron and VLDL level(Fig.4,6) was observed for the patients with malignant

tumors(0.34 ±0.09), especially with liver cancer(0.25 ±0.06). The HDL and LDL level was calculated as 0.59 ±0.10 for group 1,0.43 ±0.09 for group 2,and 0.53 ±0.10 for group 3(Fig.5).

The intensity ratio defined as ratio of the total intensity of VLDL and chylomicron to that of HDL and LDL was calculated as 0.35 ±0.14 for group 1, 0.87 ±0.38 for group 2, and 0.49 ±0.17 for group 3. Here we normalized the intensity of all components. There was a statistically significantly difference between the intensity ratios for three groups, between the total intensity of VLDL and chylomicron, and between the total intensity of HDL and LDL ($P < 0.005$, H=12.83). Three groups were most significantly separated in terms of the intensity ratio($P < 0.005$, H=19.34).

4) Standard deviation

In equation (2), σi is a standard deviation parameter for a gaussian shape. In Figs.9,10,the standard deviation values for chylomicron and VLDL are smaller than those for HDL and LDL in all groups. Cylomicron and VLDL have triglyceride as major lipid and HDL has phospholipid as major lipid. Phospholipid contains one group with elecric charge, which may confine the molecular motion in terms of electrostatic interaction This fact may cause the motion of lipid group in lipoprotein for chylomicron and VLDL to be freer than that for HDL and LDL. This well accounts for the results which is shown in Figs.9,10 from NMR theory The standard deviation value of chylomicron and VLDL was calculated as 4.43 ±0.48 Hz for group 1, 7.71 ±1.50 Hz for group 2, and 4.82 ±0.62 Hz for group 3. The standard deviation value of HDL and LDL was calculated as 14.5 ±1.3 Hz for group 1, 15.3 ±3.8 Hz for group 2,and 15.1 ±1.8 Hz for group 3.

In the present study, we could not find any statistical difference in the Fossel's test values among normal and two patients' groups. These may be due to different field intensity of NMR spectrometer and any probable experimental conditions against Fossel's.

However, we could find the analysis of spectrum through deconvolution of CH2 group's individual component may provide a different charatetristics for liver cancer group.

The elevated VLDL and chylomicron level may be due to the fact that liver is in charge of the creation or breakdown of lipoprotein, thus the change of lipid metabolism for patients with liver cancer will be much higher than the other groups. Error produced in curve fitting may be mainly due to the signal components of CH_2group.

From these results the analysis of NMR spectrum through curve fitting shows the possibility of the analytic method for the physical properties of lipoprotein in plasma. Thus this study may suggest that the spectral analysis of each isolated lipoprotein from plasma, the precise deconvolution method, are important for using the NMR spectrum on knowing information about lipoprotein.

Further clinical trials in larger populations and additional biochemical method are required to verify our present preliminary results and the possibility for diagnosis for malignant tumors

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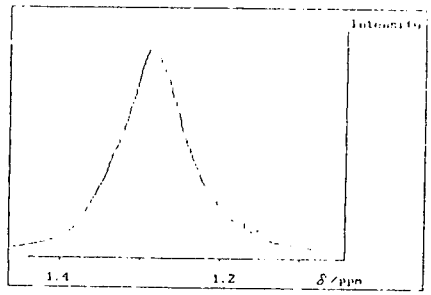

Fig.1. 300 MHz water-suppressed proton NMR spectrum of blood plasma in a patient with liver cancer: methyl group

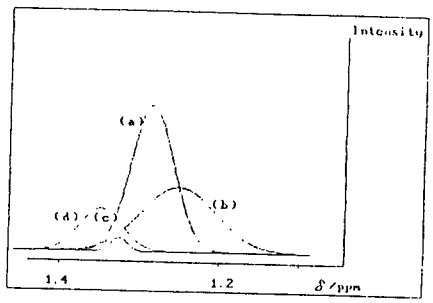

Fig.2. The individual curve-fitted spectrum
(a): VLDL and Chylomicron (b): HDL and LDL
(c): Lactate 1 (d): Lactate 2

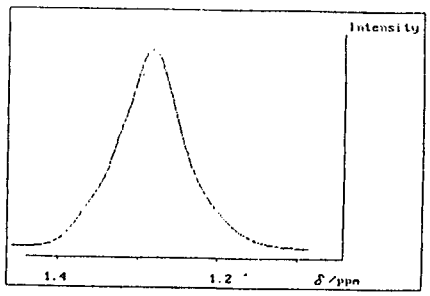

Fig.3. Reconstructed curve-fitted spectrum

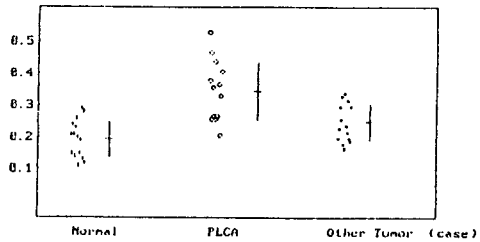

Fig.4. Intensity of VLDL and Chylomicron in the simulated spectrum;
Normal:0.19 ±0.06, PLCA:0.34 ±0.09,
and Other tumor:0.25 ±0.06
PLCA:Primary liver cancer

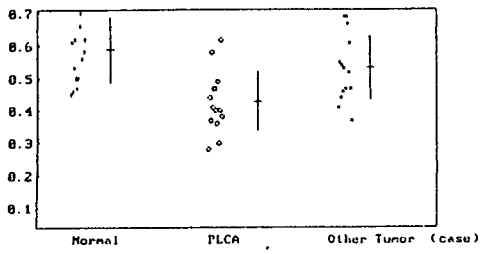

Fig.5. Intensity of HDL and LDL in the simulated spectrum;
Normal:0.59 ± 0.10, PLCA:0.43 ± 0.09,
and Other tumor:0.53 ± 0.10

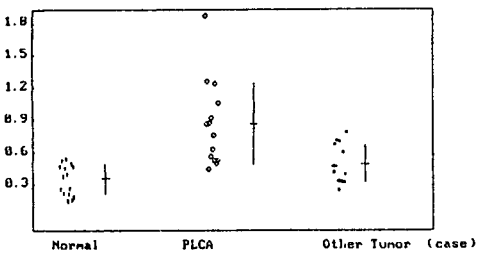

Fig.6. Intensity ratio of VLDL and chylomicron to HDL and LDL in the simulated spectrum;
Normal:0.35 ± 0.14, PLCA:0.87 ± 0.38,
and Other tumor:0.49 ± 0.17

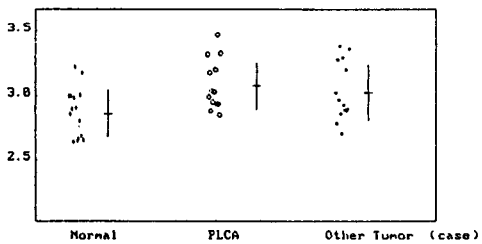

Fig.7. Moment ratio of the CH2 resonance in the simulated spectrum;
Normal:2.87 ± 0.19, PLCA:3.08 ± 0.18,
and Other tumor:3.03 ± 0.22

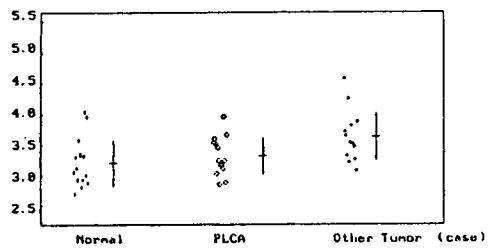

Fig.8. Moment ratio of the CH2 resonance in the raw spectrum;
Normal:3.21 ± 0.39, PLCA:3.32 ± 0.31,
and Other tumor:3.63 ± 0.19

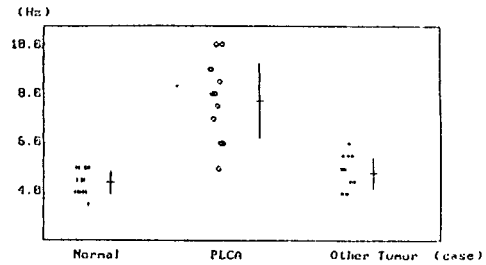

Fig.9. Standard deviation of VLDL and chylomicron in the simulated spectrum
Normal:4.43 ± 0.48, PLCA:7.71 ± 1.58,
and Other tumor:4.02 ± 0.62

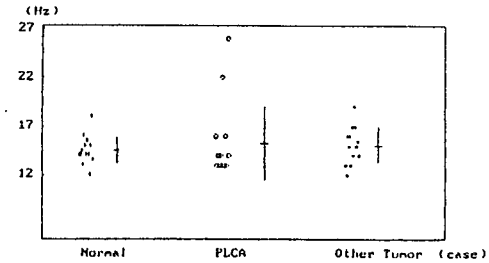

Fig.10. Standard deviation of HDL and LDL in the simulated spectrum
Normal:14.5 ± 1.3, PLCA:15.3 ± 3.8
and Other tumor:15.1 ± 1.8

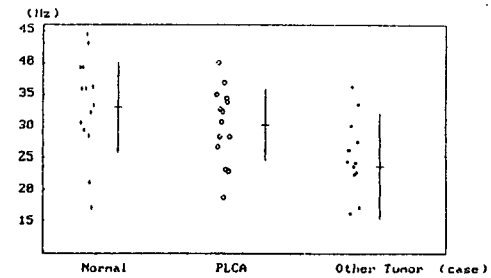

Fig.11. The mean full width at half maximum of CH2 and CH3 resonance in the raw spectrum;
Normal:32.9 ± 7.3, PLCA:38.3 ± 5.7,
and Other tumor:23.6 ± 8.4

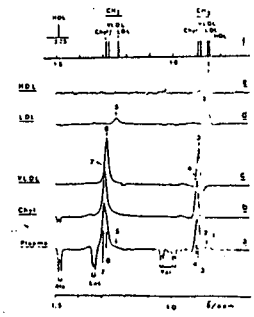

Fig.12. 500 MHz Hahn spin-echo (τ = 60 ms) ^{1}H NMR spectra of (a) blood plasma freshly and (b–e) various lipoproteins. The assignments of spin-echo peaks obtained under these conditions are illustrated diagrammatically in (f)
(Jimmy D.Bell et al., 1987).