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Acoustic Impedance Analysis with Ultra-high Frequency Ultrasound for Fatty Acid Species Identification in NASH Liver

NASH 肝臓の脂肪酸種同定のための超高周波超音波による音響インピーダンス解析

Kazuyo Ito[1‡], Kenji Yoshida[2], Kazuki Tamura[1], Jonathan Mamou[3], Hitoshi Maruyama[4], and Tadashi Yamaguchi[2] ([1]Graduate School of Engineering, Chiba Univ.; [2]Center for Frontier Medical Engineering, Chiba Univ.; [3]Lizzi Center for Biomedical Engineering, Riverside Research; [4]Graduate School of Medical, Chiba Univ)
伊藤　一陽 [1‡], 吉田　憲司 [2], 田村　和輝 [1], Mamou Jonathan[3], 丸山　紀史 [4], 山口　匡 [2]
([1] 千葉大院工, [2] 千葉大 CFME, [3] Riverside Research, Lizzi Center for Biomedical Engineering,[4] 千葉大院医)

1. Introduction

Accurate discrimination of NASH from simple steatsois (fatty liver) is a critical issue in current clinical practice because it may progress to cirrhosis or even liver cancer[1]. Although the precise mechanisms of NASH remain unclear, some reports have shown differences in the content of free fatty acid (FFA) between controls and NASH[2][3]. Thus, an ultrasound-based diagnostic technique of NASH could be developed by assessing the acoustical properties of the FFA.

Our final goal is to establish a quantitative diagnosis of NASH using ultrasonography. We focused on acoustic impedance as a possible parameter for this final goal. The aim of this study is the evaluation of the significance of the acoustic impedance measurement using a scanning acoustic microscopy (SAM) in order to investigate how the FFA influences the acoustic properties of liver cells at microscopic level as a first step.

2. Materials

Table I shows the five kinds of fatty acids and the corresponding solvent that measured by SAM first of all. These solution were made with different molar concentration from 500 mM to 1000 mM in each fatty acid. In the next step, Huh 7 cells, a human hepatoma cell, were cultured with five kinds of FFA in petri dish and fixed by formalin after culturing.

Table I Kind of free fatty acid (FFA) and solvent

Fatty acid	Solvent
Palmitate acid (PA)	methanol
Oleate acid (OA)	DEPC
Palmitoleate acid (PAOA)	methanol：DEPC = 2：1
Linoleate acid (LA)	ethanol
α linolenic acid (α-LA)	ethanol

Three pathological types (normal, NASH and cirrhosis model) of mice livers has been measured in parallel to FFA measurement. All mice were STAM™ model mice (Stelic Institute & Co.Inc., Japan). All animal protocols were approved by animal experiment committee of Chiba University.

3. Methods

In this study, acoustic impedance was calculated based on the echo amplitude analysis method relating to the pressure-reflection coefficient which was suggested by Hozumi[4][5].

A SAM system (modified AMS-50SI, Honda Electronics CO., Ltd, Japan) equipped with an 80-MHz center-frequency PVDF-TrFE transducer was employed for this acoustic impedance measurement. The spatial resolution of this transducer was 20-μm. A sample was located on a polystyrene plate and radio-frequency echo signal of 300 * 300 lines were acquired from the sample-polystyrene interface. The 2-D map of acoustic impedance in 2.4 * 2.4 mm^2 (FFA solution and cultured cells) and 4.8 * 4.8 mm^2 (mice liver) area was obtained by scanning the transducer in 16-μm steps. Three independent measurements of each samples were performed and the mean and the standard deviation of calcurated acoustic impedance values were computed. The calcurated acoustic impedance values were analyzed for statistical significance compared by one-way analysis of variance (ANOVA) followed by *post hoc* multiples comparison Tukey test using the software (IBM SPSS Statistics 22, IBM, Chicago, IL).

4. Results and discussion

Figure 1 shows the acoustic impedance of the harvested mice liver (average ± standard deviation).This result shows that the acoustic impedance of NASH is significantly lower compared to the others. On the other hand, the mean value of acoustic impedance in normal and cirrhosis liver has small difference. These results suggests that NASH liver has a different acostical property from normal liver tissue and fibers although the detailed mechanism of tissue change is still unclear.

To clarify the cause of the difference in the acoustic impedance between NASH liver and the others, futher experiments were conducted focusing on the types of fatty acid. **Fig. 2** shows the acoustic impedance of each fatty acids. **Fig.3** shows the acoustic impedance of the cultured cell with each FFA. One-way ANOVA showed the significant difference ($p < 0.01$) except for the value between the cells cultured with PA and those with LA. Especially, ANOVA revealed significant difference between all of the FFA and control. This result indicates that FFA made somewhat in the cells change, and this leads to the change of the acoustic impedance of the cells. Moreover, the cells cultured with OA have extra-low acoustic impedance compared to other cells.

We discuss the correlation between the result of the FFA solution (Fig. 2) and that of cultured cells with each FFA (Fig. 3). Especially, the point of issues are PA, OA and PAOA because they probably have significantly negative effects on the progress of NASH from simple steatosis. In case of PA, OA and PAOA, Spearman correlations between the FFA solution and cultured cells with each FFA showed the significant difference; $R^2 = 1.00$ in every concentration. This result reveals that there is no difference in the ascending order according to acoustic impedance between the result of fatty acid and that of the cultured cell in spite of the difference in absolute value. From the above, it is suggested that the PA, OA and PAOA could be distinguish with each other regardless of the condition of them.

5. Conclusion

As a basic study for the development of a quantitative diagnosis of NASH liver with the ultrasonography, the acoustic microscopy measure-ment of three types of samples (FFAs, FFAs+cells, mice liver) were performed. The measurement results of the FFA solutions and the cultured cells suggested the possibility that PA, OA and PAOA could dis-criminate from each other as an index of the acoustic impedance. Future work will focus on SAM studies at finer resolutions as well as finding means of using these results to develop and *in vivo* NASH ultra-sound diagnostic method.

Acknowledgment
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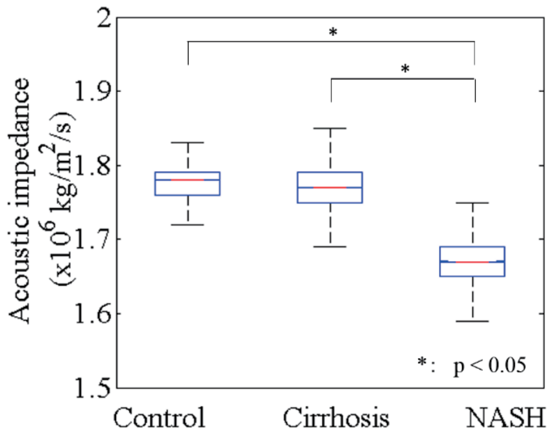

Fig. 1 Acoustic impedance in control, cirrhosis and NASH model

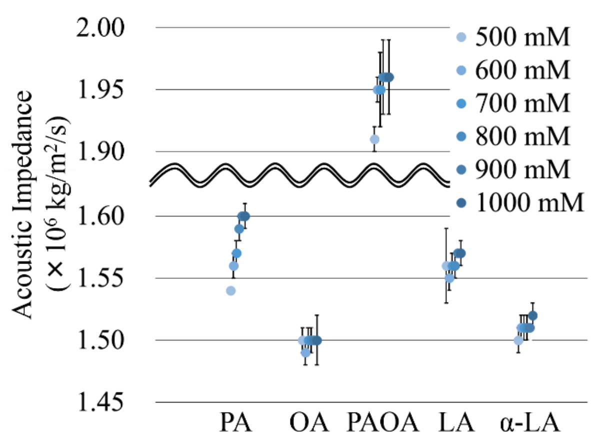

Fig. 2 Acoustic impedance of five types of FFA in solution

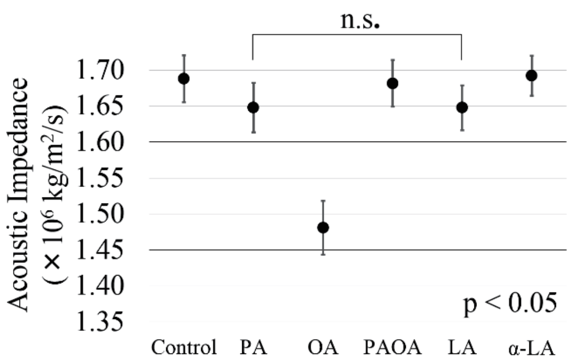

Fig. 3 Acoustic impedance of the cells with five types of FFA

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