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OPTICAL ANALYSIS OF CIRRHOTIC LIVER BY NEAR INFRARED TIME-RESOLVED SPECTROSCOPY

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ABSTRACT

The severity of liver cirrhosis was related with the optical properties of liver tissue. Various grades of liver cirrhosis were produced in rats by intraperitoneal injection of thioacetamide (TAA) for different periods: 4 weeks, 8 weeks, 12 weeks, and 16 weeks. Optical properties of the liver, absorption coefficient (μa) and scattering coefficient ($\mu s'$), were measured by near-infrared time-resolved spectroscopy. Histological examination confirmed cirrhotic changes in the liver, which were more severe in rats with TAA administration for longer periods. The μa increased in 4- and 8-week rats, and then decreased in 12- and 16-week rats. The μa of blood-free liver decreased as liver cirrhosis progressed. The hemoglobin content in the liver calculated from the μa values increased in 4- and 8-week rats and decreased in 12- and 16-week rats. The $\mu s'$ decreased in the cirrhotic liver, probably reflecting the decrease in the mitochondria content. It was shown that μa and $\mu s'$ determination is useful to assess the severity of liver cirrhosis. [S1083-3668(99)01104-1]

Keywords near-infrared spectroscopy; time-resolved spectroscopy; liver cirrhosis.

1 INTRODUCTION

In Asian countries, most cases of hepatocellular carcinoma (HCC) are associated with liver cirrhosis caused by the hepatitis virus. In cases of hepatic resection for HCC, therefore, accurate estimation of liver function is essential to prevent postoperative liver failure. In addition to conventional parameters such as nutritional status and define [indocyanine green (ICG)] clearance test, many surgeons notice from their experience that the morphology of the liver, such as color, consistency, and surface appearance, is important to assess the functional reserve. However, these findings are very descriptive and difficult to quantify.

Optical measurement using time-resolved spectroscopy (TRS) with near-infrared (NIR) light is a new technology to determine the absorption coefficient (μa) and reduced coefficient ($\mu s'$) of the tissue.[1,2] The chromophore concentration in the tissue, that is the color of the tissue, can be calculated from the μa values. It has been reported that $\mu s'$ is correlated with the mitochondrial content,[3] the fatty degeneration[4] and the solute osmolarity in the tissue.[5] Our hypothesis is that optical parameters are correlated with the histopathological changes of liver cirrhosis, which are related to a decrease in mitochondria and an increased fibrosis in the tissue.

2 MATERIALS AND METHODS

2.1 LIVER CIRRHOSIS MODELS IN RATS

Various grades of liver cirrhosis were produced in male SD rats by administration of thioacetamide (TAA) for different periods. TAA was injected into the peritoneal cavity three times per week, with a dose of 0.2 g/kg, for the initial week (normal rats) ($n=4$), 4 weeks ($n=7$), 8 weeks ($n=7$), 12 weeks ($n=5$) and 16 weeks ($n=3$). A considerable number of rats were lost from liver failure during 8 and 16 weeks.

2.2 MEASUREMENTS OF NEAR-INFRARED TIME-RESOLVED SPECTROSCOPY (NIR-TRS)

After anesthetizing the rats by intraperitoneal injection of sodium pentobarbital (50 mg/kg), the liver was exposed for optical measurement.[5]

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Time-resolved measurements were done using a prototype instrument of Hamamatsu Photonics [full width at half maximum (FWHM)=140 ps]. For *in vivo* measurement, the optical fibers were placed on the liver surface in the reflectance geometry. The fiber separation was 1.7 cm. The wavelengths employed were 780 and 830 nm. Obtained time profile spectra were digitized and fitted to the diffusion equation of the semifinite model as reported by Patterson, Chance, and Wilson.[2] The measurements were done three times, and the average values of μa and $\mu s'$ were used. After *in vivo* measurements, the liver was removed from the abdomen, and the blood in the liver was washed out by normal saline, in order to determine the μa of blood-free liver using the matching method.[6] After TRS measurements, the liver was histologically examined. Hemoglobin saturation (SO_2) and hemoglobin content (THB) of the liver were calculated as

$$\mu a_{\text{blood}} = \mu a_{\text{liver}} - \mu a_{\text{blood-free liver}},$$

$$\mu a_{\text{blood}} = \mu a_{\text{Hb}}[\text{Hb}] + \mu a_{\text{HbO}_2}[\text{HbO}_2],$$

where $\mu a_{\text{Hb}780} = 2.567$, $\mu a_{\text{HbO}_2 780} = 1.710$, $\mu a_{\text{Hb}830} = 1.813$, and $\mu a_{\text{HbO}_2 830} = 2.441$ (natural log),

$$SO_2(\%) = [\text{HbO}_2]/([\text{HbO}_2]+[\text{Hb}]) \times 100,$$

$$\text{THB(mM)} = [\text{HbO}_2]+[\text{Hb}].$$

Data were expressed as mean$\pm$SD($\log_{10}$). Statistical analysis was done using STATVIEW (Macintosh computer). Statistical significance was considered when $p < 0.05$.

3 RESULTS

Grades of liver cirrhosis were more severe in the rats with longer administration of TAA (Figure 1).

The μa of the liver (data at 780 and 830 nm in cm^{-1}) increased from 0.295$\pm$0.027 and 0.277$\pm$0.017 to 0.321$\pm$0.035 and 0.310$\pm$0.022 in the 4-week rats and 0.313$\pm$0.056 and 0.289$\pm$0.039 in the 8-week rats, respectively. Then the μa decreased to 0.225 $\pm$0.051 and 0.216$\pm$0.033 in the 12-week rats and 0.250$\pm$0.017 and 0.247$\pm$0.018 in the 16-week rats, respectively (Figure 2). The μa of the blood-free liver decreased as cirrhotic changes progressed; 0.185$\pm$0.009 and 0.167$\pm$0.008 in the normal rats, 0.164$\pm$0.031 and 0.141$\pm$0.015 in the 4-week rats, 0.152$\pm$0.019 and 0.133$\pm$0.012 in the 8-week rats, 0.138$\pm$0.018 and 0.130$\pm$0.014 in the 12-week rats, and 0.136$\pm$0.012 and 0.125$\pm$0.007 in the 16-week rats, respectively. THB increased in the 4- and 8-week rats, but returned to normal levels in the 12- and 16-week rats (Figure 3). SO_2 did not significantly change in the cirrhotic livers (Figure 4). The corresponding $\mu s'$ of the liver (in cm^{-1}) decreased in the cirrhotic livers from 4.89$\pm$0.57 and 4.80$\pm$0.45 in the normal rats to 4.70$\pm$0.73 and 4.77$\pm$0.45 in the 4-week rats, 4.14$\pm$0.57 and 4.09$\pm$0.56 in the 8-week rats, 4.08$\pm$0.72 and 4.19$\pm$0.51 in the 12-week rats, and 4.00$\pm$0.34 and 3.90$\pm$0.15 in the 16-week group, respectively (Figure 5).

4 DISCUSSION

Among NIR spectroscopy which has been applied widely in the biomedical field, time-resolved measurement is a new technology to determine μa and $\mu s'$ of the tissue. It has been shown that μa gives chromophore concentration and $\mu s'$ contains information about subcellular events in the tissue.[7] Our previous studies were aimed at the application of NIR and NIR-TRS to liver transplantation, especially examining the graft liver with fatty change.[4,8] In this study, we investigated the application of NIR-TRS to liver resection of hepatocellular carcinoma associated with liver cirrhosis. Quantification of cirrhotic changes should increase the safety of surgery and reduce postoperative liver failure.

Liver cirrhosis of rats produced by TAA is an established model resembling human liver cirrhosis.[9] It has been reported that fibrotic changes in the liver tissue gradually increased as the administration period was prolonged until 20 weeks. We also confirmed period-related cirrhotic changes by histological examination.

The μa of the cirrhotic liver increased in the early stage, then decreased when the cirrhotic change progressed. In contrast, the μa of blood-free livers (mitochondrial content) decreased in the cirrhotic liver depending on the periods of TAA administration. The μa of *in situ* liver principally represents the hemoglobin and mitochondrial contents.[10] Therefore, it is clear that the mitochondrial content decreased in the cirrhotic liver depending on severity. Hemoglobin content in the liver, which was calculated from the μa values of the *in situ* liver and blood-free liver, increased in the liver with relatively mild cirrhosis, but decreased as the cirrhotic changes worsened.

The $\mu s'$ of the cirrhotic liver decreased as cirrhotic changes progressed. From previous studies, the decrease in $\mu s'$ is expected to reflect a decrease in the mitochondrial content.[3] This interpretation is compatible with the changes in μa of blood-free cirrhotic liver, but the decrease in $\mu s'$ was not so marked as the changes in μa of blood-free liver. The effects of increased collagen fibers and infiltrated inflammatory cells in the tissue are not known. The osmolarity of the intracellular and extracellular fluid may be changed and influence the $\mu s'$ of the liver.[5] Further investigation is required to clarify the biological meaning of $\mu s'$ in the cirrhotic liver.

In conclusion, (1) μa increased in mild cirrhotic liver, but decreased as cirrhosis progressed. The first increase in μa is caused by the increase in the

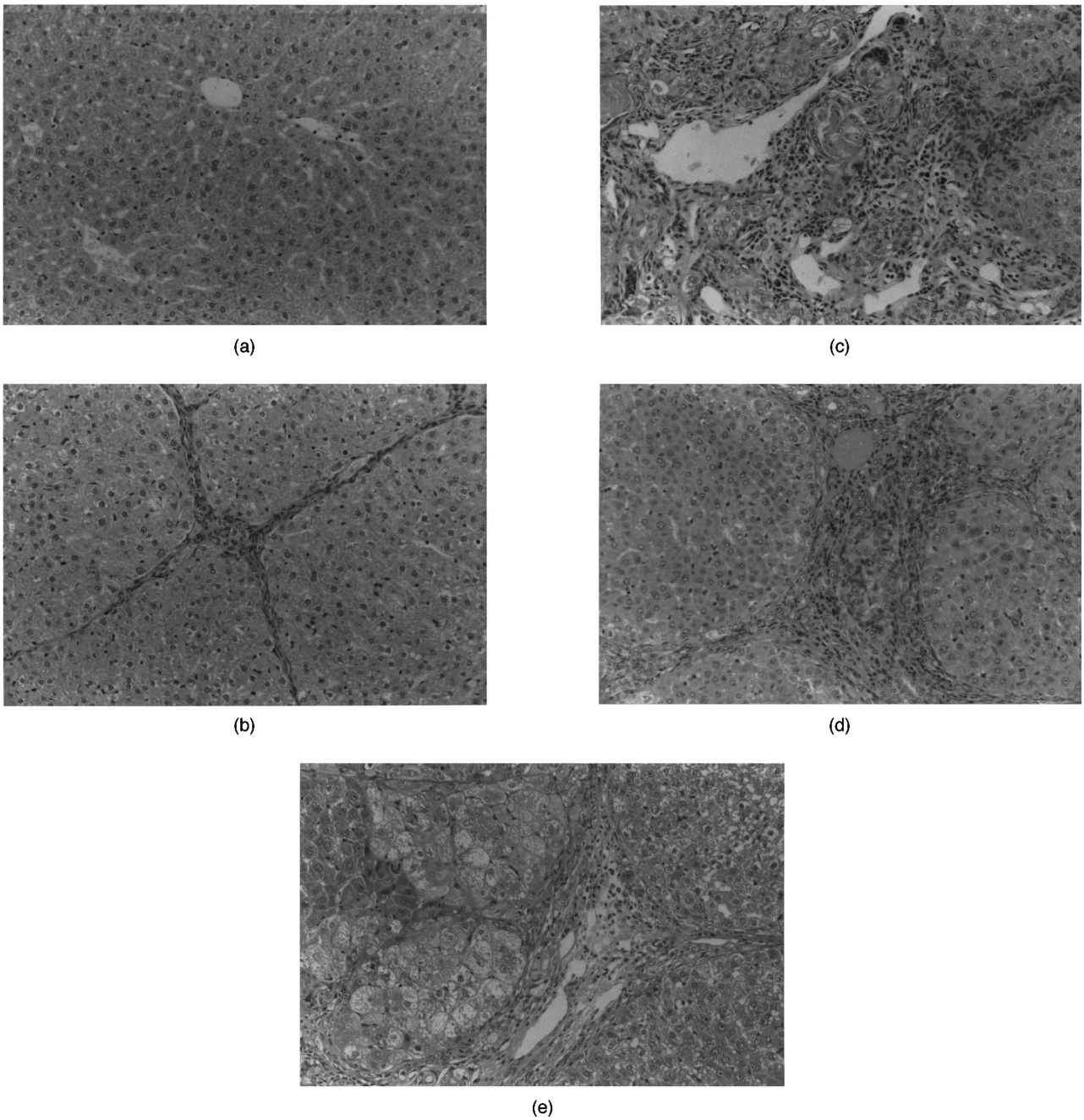

Fig. 1 (a) normal (×100); (b) 4 weeks: the fibrosis was thick and bridging performed (×100); (c) 8 weeks: the fibrosis is more spread (×100); (d) 12 weeks: there is spread proliferation of the bile duct in the bridge formation (×100); (e) 16 weeks: invasion of inflammatory cells with interstitial edema was seen. Small vessels around the portal vein were also observed (×100).

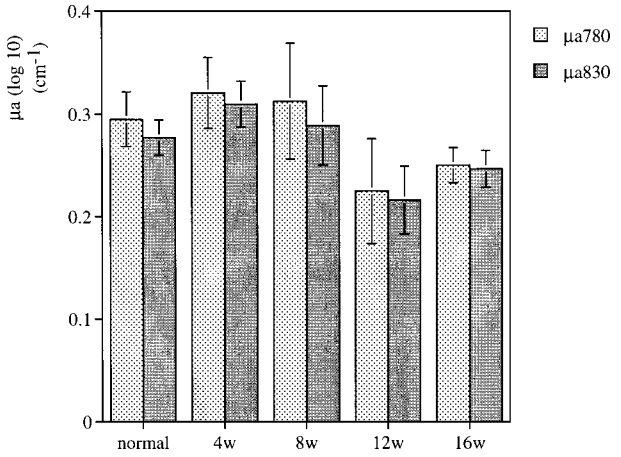

Fig. 2 Changes in μa of the cirrhotic liver. The μa increased in the 4- and 8-week groups, and decreased in the 12-week and 16-week groups. ANOVA showed significant difference at 780 and 830 nm. Scheffe's test showed significant difference with 4-week vs 12-week and 8-week vs 12-week groups at 780 and 830 nm.

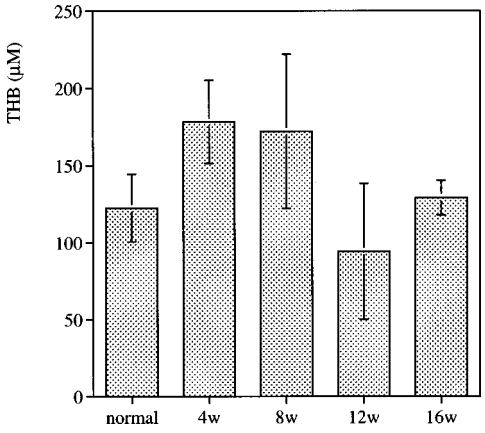

Fig. 3 Changes in the hemoglobin content in the liver. Hemoglobin content increased in mild cirrhosis, but decreased as the cirrhotic changes became severe. ANOVA showed significant difference. Scheffe's test showed significant difference with 4-week vs 12-week and 8-week vs 12-week groups.

blood in the liver, and the second decrease was due to the decrease in the cytochrome oxidase. (2) The $\mu s'$ decreased in accordance with the severity of liver cirrhosis, probably reflecting the decrease in mitochondrial content inside the liver. (3) This study indicates that the clinical application of TRS-NIR is encouraging.

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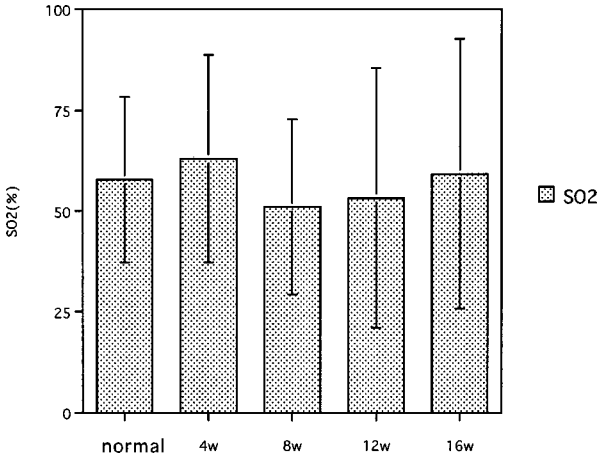

Fig. 4 Changes in the hemoglobin saturation (SO) in the liver. ANOVA did not show significant difference.

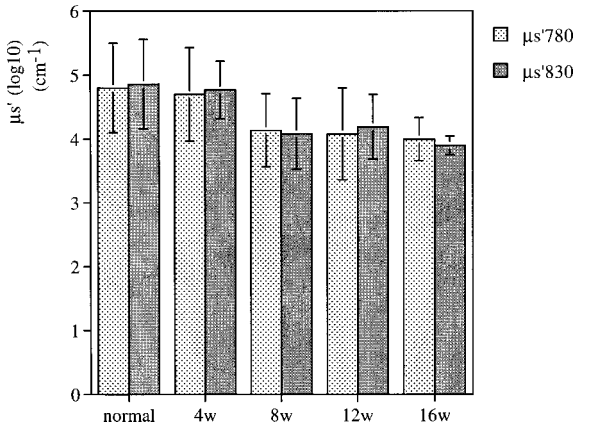

Fig. 5 Changes in $\mu s'$ of the cirrhotic liver. The $\mu s'$ decreased in the cirrhotic livers. ANOVA showed significant difference at 830 nm.

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