Non-invasive and quantitative evaluation of hepatic fat accumulation in ob/ob mice

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Introduction: Alcoholic and nonalcoholic fatty livers are highly prevalent in human populations and may develop into steatohepatitis and in some cases into cirrhosis requiring liver transplantation. AMP-activated protein kinase (AMPK) has become as a novel therapeutic target for the treatment of metabolic syndromes. We have isolated a novel AMPK pathway activator cryptotanshinone from *Salvia militorrhiza*, which is an herb that is used extensively in Asian medicine and that is known to exert beneficial effects on the circulatory system. ^{1}H magnetic resonance spectroscopy has a high sensitivity for assessing the amount of hepatic fat content. The purpose of this study was to non-invasively and quantitatively evaluate and monitor the hepatic fat accumulation in *ob/ob* mice depending on the treatment period or amount of cryptotanshinone by using ^{1}H-MRS technique.

Materials and Methods: Sixty *ob/ob* mice (C57BL6.V-*Lep*^{ob}/J, SLC Japan, 6 to 14 week-old) and mice (C57BL6/J, 6 week-old) underwent multi-slice T1-weighted MRI and liver ^{1}H-MRS on a 4.7 T animal MR scanner (Biospec, Bruker). Hepatic lipid ratios (HLRs) in control and cryptotanshinone administered *ob/ob* mice were weekly measured using the MRS technique. Starting at 12 weeks of age, cryptotanshinone (0, 100, 200, 400, 600, and 800 mg/kg/day; 10 animals per group) was daily administered for 2 weeks, and the HLRs were measured at 13 and 14 weeks of age. T1-weighted images were obtained to study the distribution of fat stores in *ob/ob* mice, using the following parameters: TR/TE = 665/14 ms; slice thickness = 1 mm; FOV = 3.5 × 3.5 cm^2; matrix = 256 × 256. ^{1}H localized spectra were acquired from the left and right lateral lobes of the liver using a stimulated echo sequence with TR/TE = 2500/144 ms, voxel size = 3 × 3 × 3 mm^3, and NEX = 64. The HLR in liver was measured using the following formula: *HLR = 100 × [integral value of all lipid peaks]/[integral value of water peak]*. After the MRI and MRS experiments, the mice were sacrificed and perfused transcardially with PBS, and then livers were removed. The livers were fixed with 4% paraformaldehyde, and oil red staining was performed according to a standard protocol.

Results: On T1-weighted MR images, the liver signal intensity of *ob/ob* mice was higher than that of normal mice, and increased with age in *ob/ob* mice. Two weeks after cryptotanshinone treatment, the liver signal intensity was decreased in a dose-dependent manner. The oil red staining also showed that the intrahepatic lipid content was decreased with the treatment dose of cryptotanshinone in a dose-dependent manner. The measured *in vivo* HLRs were in good correlation with the histopathologic findings (Figure 1). While the mean HLR was 13% in wild type mice, the value was higher in *ob/ob* mice: it was 48% in 6 weeks *of age*, and increased up to 95% at 12 weeks of age, then 105% at 14 weeks of age. After two weeks treatment of the cryptotanshinone, it was significantly decreased to 50 ± 5 % in 800 mg/kg group, 63 ± 3 % in 600 mg/kg group, 65 ± 3 % in 400 mg/kg group, and 86 ± 3 % in 200 mg/kg group, while it was 107 ± 19 % in 100 mg/kg group. Thus compared with control group (121 ± 5), the treatment effects were significant at the doses higher than 200 mg/kg (Figure 2).

Conclusion: Our results clearly demonstrate that *in vivo* ^{1}H-MRS can be an extremely valuable technique for longitudinal quantitative evaluation and monitoring of hepatic fat accumulation in mice. They show a good correlation with hepatic oil red staining results, providing important insights into the genetic, environmental, and dietary factors affecting fat deposition and accumulation within the mouse liver. This technique can be an assessment tool in the development of novel therapeutic approaches for the treatment of human fatty liver disease, especially for non-invasively evaluating the clinical efficacy of a fatty liver drug candidate without biopsy. Here we also elucidated the efficacy of cryptotanshinone as a novel therapeutic drug candidate for the treatment of fatty liver disease using ^{1}H-MRS technique in *ob/ob* mice.

References

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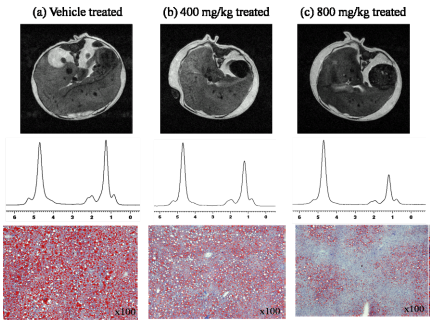

Figure 1. The corresponding results of MRI, MRS, and histopathological findings are shown from vehicle (a), 400 mg/kg (b) and 800 mg/kg (c) groups after two weeks administration of cryptotanshinone.

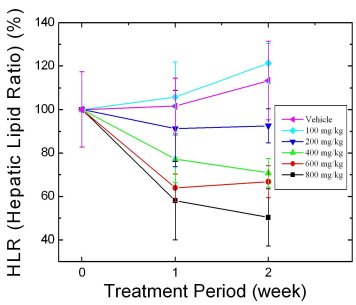

Figure 2. Dose dependent HLRs are shown after the cryptotanshinone treatment to *ob/ob* mice for two weeks (from 12 to 14 weeks of age). Treatment effects are significant at the dose higher than 200 mg/kg.