Cannabinoid-1 Inverse Agonist Treatment and Cessation Effects on Intrahepatocellular Lipid and Adipose Tissue Distribution in an Obesity Rodent model

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Introduction

Cannabinoid receptors type 1 (CB1r) in the central nervous system are involved in appetite modulation. CB1r antagonist treatment results in food intake suppression and body weight loss, accompanied by metabolic improvements in animals and man. In this study, the aim was to describe *in-vivo* the changes in intrahepatocellular lipids (IHCL), total, intra-abdominal and subcutaneous white adipose tissues (TAT, IAT, SAT) and body weight (BW) during treatment with the CB1 antagonist rimonabant (Sanofi-aventis, France) and following cessation of treatment in a mouse obesity model which resembles human obesity. Specifically we asked the following question: does interruption of treatment result in a rapid rebound of intrahepatocellular lipids?

Method

Female C57Bl/6J mice (n = 36) were pre-treated with high fat diet (HFD) for twelve weeks and during that period a MRI baseline session was performed. The mice were then treated daily for 21 day with rimonabant (20µmol/kg) administered by oral gavage. After treatment cessation, mice were monitored for a further 22 day period. Mice were imaged at days 1, 4, 7, 12 and 20 during the treatment phase and at days 28, 32 and 42 after treatment cessation.

MRI/S was carried on a 9.4T/20 USR Bruker Biospec scanner. Animals were anaesthetized using isoflurane and placed in a 50 mm resonator (m2m Imaging, Brisbane, Australia). The protocol (30 min per animal) consisted of: (i) acquisition of three sets of respiratory gated high resolution 3D FISP scans with flip angles α:[4°, 20°, 45°], TR/TE: 3.3ms/1.7ms, field of view: 100x45x45 mm and matrix size: 428x192x192, and (ii) acquisition of a localized PRESS ^{1}H spectra obtained from a 2x2x2 mm^3 voxel in the liver for IHCL measurement, TR: 3 s, TE: 6.7 ms, SW: 4006 Hz, 64 averages, and 2048 data points. Areas of water peak (A_w) at 4.7 ppm and lipid methylene (CH_2) peak (A_f) at 1.3 ppm were measured and IHCL expressed in percent as: IHCL = 100 x A_f / (A_w+A_f). 3D images were evaluated using a fully automatic in-house procedure for body fat segmentation (Figure 1a-c). Post-processing took less than 30s per animal. At each time point the mass of each adipose compartment was measured. Results are expressed as mean ± SEM. One-way analysis of variance was used to compare baseline to treatment effects.

Results

During the first twelve weeks of fat feeding BW increased from 20 ± 0.3 g to 42.7 ± 0.7 g. After 21 days of rimonabant treatment there were significant reduction in body weight (42.7 ± 0.7 g to 32.8 ± 1.5 g, $p < 0.01$), IHCL (20 ± 0.4% to 8 ± 0.8%, $p < 0.001$), TAT (19.0 ± 0.4 g to 10.9 ± 1.1 g, $p < 0.001$), IAT (7.0 ± 0.2 g to 3.7 ± 0.4 g, $p < 0.001$), and SAT (12.0 ± 0.2 g to 7.2 ± 0.6 g, $p <$ 0.001) compared to baseline. As shown in figure 1d the responses were in a time dependent manner and the fastest decrease occurred during the initial period of treatment (day 0 to day 12). During the final treatment period (day 12 to day 20), the improvement of all measured variables remained unchanged.

One week after interruption of treatment, a striking rebound of IHCL level was seen (from 8 ± 0.8% at day 20 to 21 ± 1.1% at day 28) and a progressive weight regain took place. At the end of the weight regain period (day 42) IHCL as well as TAT, IAT, SAT and BW returned to near their respective baseline levels.

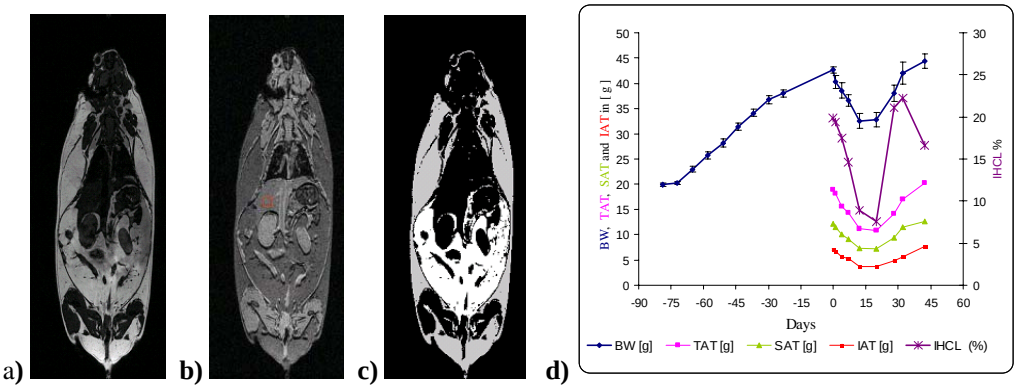

Figure1: Selected slice from a 3D MRI data set acquired with a flip angle α= 45° (a) and the corresponding slice from the data set acquired at α= 4° (b). The red square in the liver represent the position of the voxel used for localized ^{1}H spectroscopy. Corresponding slice showing IAT and SAT compartments after automatic segmentation (c). Effect of rimonabant on %IHCL, TAT, IAT, SAT and BW as measured at each imaging time point (d). Note the second y-axis for %IHCL.

Conclusion

In conclusion, the present *in-vivo* MRI/S efficacy study has shown that treatment with the CB1 antagonist rimonabant reduced body weight and adiposity in our obesity mouse model. Interruption of treatment resulted in a marked increase in IHCL and a regain in, IAT, SAT, TAT and BW. These results were obtained by designing an animal setup and imaging protocol allowing us to produce high resolution 3D whole body mice images optimized for both automatic body composition and intra-hepatic lipid level measurements. Furthermore, the high throughput of the acquisition and analysis was a prerequisite for the longitudinal evaluation of novel pharmaceutical agents designed to correct metabolic disorders.