TIME COURSE OF PPARA AGONIST EFFECTS ON PLASMA LIPID LEVEL, LIVER SIZE AND HEPATIC LIPID CONTENT IN FAT FED RATS: AN IN-VIVO MR IMAGING AND SPECTROSCOPY STUDY

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Introduction

Peroxisome proliferator–activated receptor alpha (PPARα) is a ligand-activated transcription factor and member of the nuclear hormone receptor superfamily that regulates the metabolism of glucose and lipids [1]. PPARα is pre-dominantly present in the liver. In rodents pharmacologic activation is known to reduce plasma triglyceride levels (TG), increase hepatic fatty acid oxidation and cause hepatomegaly.

The primary aim of this study was to explore the relationship between the time dependent effects of the PPARα agonist WY 14,643 on plasma TG levels, hepatic lipid content and volume in fat-fed Sprague-Dawley rats. An estimate of liver mass (LM) and total intrahepatocellular lipid mass (t-IHCL) were obtained non-invasively at three time points in each experimental animal by means of magnetic resonance imaging/spectroscopy (MRI/S). At corresponding times blood samples were collected for analysis of clinical chemistry variables in plasma. LM and t-IHCL estimates obtained *in-vivo* at the final imaging occasion were compared respectively to post-mortem liver weight and hepatic lipid measured by an enzymatic/colorimetric method.

Method

Two groups of high-fat fed male Sprague Dawley rats (N=8/group) were studied. Following a baseline MRI session one group was treated daily with WY 14,643 (30µmol/kg/day) by oral gavage while the other received vehicle. Additional imaging sessions were performed at days two and twenty eight after treatment start.

MRI/S was carried on a 4.7T/40 cm Bruker Biospec MR scanner. Animals were anaesthetized using isoflurane and placed supine in a 72 mm resonator. Liver volume was measured using two sets of respiratory gated high resolution segmented 3D FISP scans with different flip angles (α: 5° and α: 15°), TE/TR: 4.2ms/2.1ms field of view: 50x65x65 mm and matrix size: 128x128x128. Liver volume was quantified using a semi-automatic segmentation procedure based on multi-spectral classification using the software package Analyze 6.0 (Figure 1). Prior to segmentation, each 3D volume was subsampled by a factor of ten in the coronal plane to reduce the segmentation procedure. A pre-study showed that this subsampling was possible without significant loss of accuracy. A density factor of 1.06 g/ml was used to convert liver volumes into LM. Respiratory gated localized ^{1}H spectra were measured on a 64 µl voxel in the liver using a PRESS sequence (TR: 3 s, TE: 6.7 ms, SW: 4006 Hz, 64 averages, and 2048 data points). Water and lipid methylene $(CH_2)_n$ peak areas were calculated by Lorentzian/Gaussian curve fitting using XWIN-NMR. From the liver fat percent estimated using the lipid to water ratio and LM an estimate of t-IHCL was obtained at each time point. Statistical comparisons of plasma and *in-vivo* variables were made in a linear mixed model. Results are expressed as mean ± SEM.

Results

It was found that fat feeding raised plasma TG levels from 0.7±0.1 to 2.8±0.2 mM. After only 2 days of treatment, WY 14,643: reduced plasma TG to 0.8±0.1 mM, and increased t-IHCL and LM by 200% and 28%, respectively. At day 28 this reduction in plasma TG was maintained while there were further significant increases in both t-IHCL and LM compared to day 2. It is worth noting that, unlike LM, the increase observed in t-IHCL from day 2 to day 28 in the treated group was not significantly different from the increase measured in the control group (Figures 2a, 2b). MRS estimates of t-IHCL on day 28 correlated well with liver triglyceride content measured ex-vivo by biochemistry ($r = 0.84$, $p < 0.01$). Furthermore LM correlated well with liver mass determined by weighing ($r = 0.97$, $p < 0.01$, Figures 2c, 2d).

Conclusion

In conclusion, the PPARα agonist WY 14,643 corrects the hypertriglyceridemia caused by high fat feeding with a rapid and sustained action. MRI/S revealed an accompanying expected increase in liver size. In addition and unexpectedly the liver lipid pool was actually increased. Thus MRI/S is a useful tool to probe the time dependant effect of drugs on hepatic lipid content and volume. A robust protocol has been developed to measure liver volume and liver triglyceride content in-vivo and at multiple time points. A segmentation procedure was also developed allowing rapid segmentation of the liver.

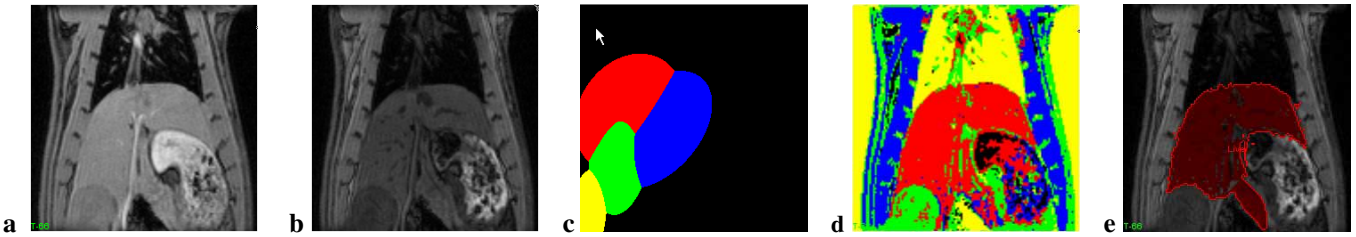

Figure 1: Selected slice from a 3D data set acquired with a flip angle α=5° (**a**) and the corresponding slice from the data set acquired at α=15° (**b**). Manually defined classes were traced on the image pair allowing Gaussian Maximum Likelihood classification of the corresponding 2D score plot (**c**). The resulting classifier was applied to the image volume for 3D segmentation of the liver and other tissues (**d**). The liver was then extracted and edited manually (**e**).

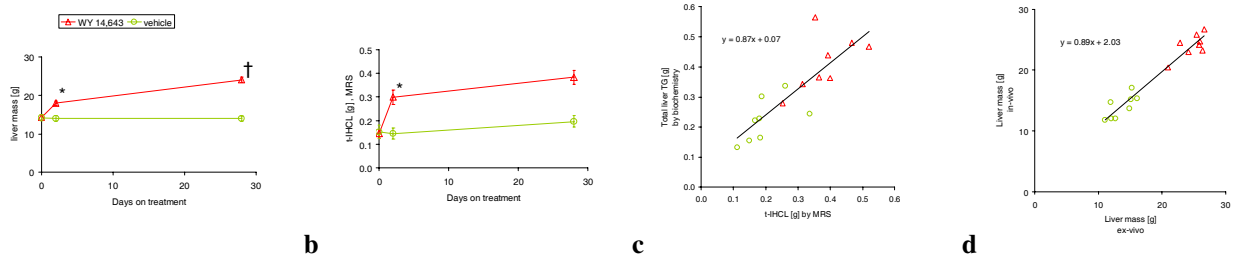

Figure 2: Liver mass (**a**) and total intrahepatocellular lipid (**b**) plotted versus time. Correlation of liver triglyceride content measured ex-vivo by an enzymatic/colorimetric method versus t-IHCL on day 28 measured by MRS (**c**) and correlation of liver mass measured in-vivo versus liver mass determined by weighing (**d**). * $p<0.05$ for $WY14{,}643_{day2}$ - $WY14{,}643_{day0}$ vs $Vehicle_{day2}$ - $Vehicle_{day0}$. † $p<0.05$ for $WY14{,}643_{day28}$ - $WY14{,}643_{day2}$ vs $Vehicle_{day28}$ - $Vehicle_{day2}$.

Reference: 1. F. Blaschke. Obesity, peroxisome proliferator-activated receptor, and atherosclerosis in type 2 Diabetes, Arterioscler Thromb Vasc Biol. 2006;26:28-40.