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Endocannabinoid receptor blockade increases hepatocyte growth factor and reduces insulin levels in obese women with polycystic ovary syndrome.

Running title: CB1 blockade and cytokine effects in PCOS

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There is evidence from animal and in-vitro studies that activation of the endocannabinoid system (EC) through cannabinoid receptor 1 (CB-1) is associated with liver injury, inflammation and hepatocellular carcinoma.[1] Data suggests endogenous cannabinoids (EC) are related to fatty liver metabolism with a role in non-alcoholic fatty liver disease (NAFLD) through modulating lipid metabolism that may be ameliorated by CB1 receptor antagonism with rimonabant.[2] This is of particular importance as NAFLD is the most common cause of chronic liver disease with liver dysfunction leading liver cirrhosis. The diagnosis of NAFLD can only be confirmed by a liver biopsy, as liver enzymes such as alanine aminotransferase (ALT) used, as a serum marker may not be elevated.

NAFLD has increased prevalence in women with polycystic ovarian syndrome (PCOS) the most common endocrine problem in premenopausal women. Insulin resistance is common in PCOS, which exacerbates fatty acid oxidation leading to increased hepatic stress and liver injury. Metformin, an insulin sensitising agent effective in treating PCOS and with benefit in NAFLD.

Studies to investigate the effect of rimonabant/endocannabinoid receptor blockade on the liver are no longer possible as rimonabant was withdrawn from the market because of increased neuropsychiatric side effects. However, existing studies can give an indication as to whether future CB1 antagonists may have value in the treatment of NAFLD. We hypothesized that in PCOS – a condition associated with low grade inflammation – that even in the absence of overt liver dysfunction hepatic CB1 blockade would reflect in improved pro-inflammatory cytokine changes, particularly hepatocyte growth factor (HGF), that are implicated in hepatic injury.

We performed a post hoc analysis on a randomized open labelled parallel study with metformin (500mg three times daily) or rimonabant 20mg daily (licensed at that time) in 20 PCOS patients for 3 months, described in detail previously[3] (Clinical trial registration ISRCTN75758249).

The Bio-Plex 200 system with HTF (Bio-Rad, Hercules, CA) was used to evaluate the sera and the levels of HGF, IL-1β, IL-6, IL-7, IL8, IL-10, IL12, TNF-α, MCP-1 and INF-γ and compared to a set of standards that were run simultaneously in the assay [4].

Details of the power calculations and statistical methods are detailed previously[3].

All 20 subjects recruited completed the 3-month study period. The compliance was 98% in both groups by counting returned medication.

The patients were weight matched in each group (Table 1).

As detailed previously weight, waist circumference, free androgen index (FAI), insulin resistance (IR) reduced significantly after 12 weeks of rimonabant treatment ($p<0.01$).

Hepatocyte growth factor was increased by rimonabant (396 ± 124 vs. 474 ± 166 pg/ml, $p<0.05$), unchanged with metformin treatment and correlated with post treatment insulin ($p<002$, $r=0.59$) and HOMA-β ($p<0.05$, $r=0.63$). HGF did not correlate with any clinical parameters. The pro-inflammatory cytokine profiles IL-1β, IL-6, IL-7, IL8, IL-10, IL12, TNF-α, MCP-1 and INF-γ did not differ following rimonabant or metformin treatment (Table 1).

The original study showed a decrease in weight, testosterone and IR with rimonabant in comparison to metformin. This analysis showed that rimonabant increased HGF which in turn correlated with a reduction in beta cell function as assessed by HOMA-β, but did not alter the pro-inflammatory cytokine profile that has been associated with liver inflammation

and malignancy. HGF has been shown to increase beta cell mass and to be related to insulin resistance though the underlying mechanisms are unclear, [5] and in this context may well have been stimulated directly by EC receptor blockade. Serum HGF levels may be altered in weight gain and elevated levels are related to body mass index and waist circumference as shown in a cross sectional study in subjects with metabolic syndrome; however, the change in HGF in this study was independent of those clinical parameters. There is evidence to suggest that a fall in HOMA-β is secondary to a fall in insulin resistance; however, the changes in HGF and HOMA-β, and their correlation were only seen in the rimonabant not the metformin group (where in both insulin resistance fell) suggesting a direct effect of rimonabant.

The EC system comprises of CB1 (in the brain and the peripheral tissues) and CB2 receptors in the immune and endothelial system. EC receptor expression is low in the healthy liver; however, once the liver is subjected to injury, the EC system becomes dysregulated and can be detected in the early stages of liver injury[2]. EC system has been recently suggested as a novel mediator of liver disease supported by animal and in vitro studies that activation of the EC system through CB1 is associated with enhanced liver injury, inflammation and hepatocellular carcinoma.[1] Further data suggests endogenous cannabinoids (EC) are closely related to fatty liver metabolism through the CB1 receptors and have a role in NAFLD through modulating lipid metabolism[2]. Animal studies show that treatment with rimonabant decreased expression of CB1 receptors in diet induced obese mice and was shown to decrease steatosis along with associated metabolic diseases. In the four large human trials, rimonabant was shown to have a significant effect on weight reduction, liver

steatosis and insulin sensitivity. These data therefore support the careful reevaluation of rimonabant as therapy for NAFLD particularly given the paucity of effective therapy for this condition.

The pro-inflammatory cytokine profile was unchanged with either rimonabant or metformin treatment, probably reflecting that these subjects had normal liver function and low background inflammation confirmed by a normal CRP. However, others have shown that weight loss of exactly the same amount of 6.1kg did have a beneficial effect in obese subjects with a similar cytokine profile, though in this study patients were treated with sibutramine that may have been responsible for the changes rather than weight loss *per se*.

In conclusion, rimonabant had a beneficial effect on insulin resistance and showed an increased HGF that was related to both decrease in insulin resistance and beta cell function in obese women with PCOS without liver disease, independent of weight loss, and likely to be a direct effect on the CB1 receptors that is in accord with the increasing evidence from animal and in vitro data.

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Table 1: Comparison of cytokine profile before and after 12 weeks treatment with metformin or rimonabant.

Parameter	**Metformin group (n=10)**			**Rimonabant group (n=10)**		
	Baseline	**12 weeks**	***p*-value**	**Baseline**	**12 weeks**	**P value**
HGF	329±144	333± 133	0.65	396 ±125	474±166	0.05[a]
IL-1β	86±56	105± 89	0.17	85± 77	91± 91	0.32
IL-6	12± 6	13± 7	0.31	17± 8	18± 9	0.31
IL-7	249± 18	252± 31	0.69	235± 47	263± 49	0.11

IL-8	12± 13	11± 14	0.89	7± 11	18± 13	0.38
IL-10	23± 32	27± 30	0.29	10± 6	10± 6	0.92
IL-12	203± 60	223± 83	0.11	201± 41	207± 33	0.50
TNF-α	7± 3	8± 4	0.33	9± 4	9± 4	0.99
MCP-1	294± 66	298± 88	0.80	279± 60	307± 58	0.6
INF-γ	30± 10	30± 9	0.83	30± 7	36± 9	0.6

HGF= Hepatocyte Growth Factor, IL-1β = Interleukin-1Beta, IL-6= Interleukin-6, IL-7= Interleukin-7,

IL-8= Interleukin-8, IL-10= Interleukin-10, IL-12= Interleukin-12, TNF-α = Tumor Necrosis Factor Alpha,

MCP-1= Monocyte Chemotactic Protein-1, INF-γ = Interferon-Gamma.

Data are presented as mean ± SEM. ***a:*** Significant difference from baseline.