**ABSTRACT**

Farnesol is a 15-carbon isoprenoid alcohol, and its metabolites, collectively known as “farnesoids,” modulate metabolic processes in rodent liver through activation of nuclear receptors such as peroxisome proliferator-activated receptor alpha (PPARα), constitutive androstane receptor (CAR) and farnesoid X-receptor (FXR). However, the effects of farnesol on human hepatic lipid metabolism have not been investigated. Therefore, the aim of this study is to determine whether farnesol modulates hepatic pathways associated with human lipid homeostasis. For these studies, we selected the HepaRG cell line, a bipotent progenitor cell line that can be differentiated into hepatocyte-like and hepatic progenitor cell lines. We hypothesized that farnesol might regulate the expression of genes involved in human hepatic lipid metabolism by altering the activity of the lipid-sensing nuclear receptors, PPARα, CAR, and FXR. Because CAR signaling is a phenotype that is associated with differentiated hepatocytes, we evaluated the intactness of CAR signaling as a test of the suitability of HepaRG cells for our studies. Treatment of differentiated HepaRG cells with the known CAR activators, C7 and phenobarbital, strongly increased expression of the CAR target gene, CYP2B6, and farnesol treatment also increased CYP2B6 expression. We are currently performing studies to evaluate functionality of the FXR and PPARα pathways and to test farnesol’s ability to modulate them. Additionally, the HepaRG cells are being treated with the free fatty acids oleate and palmitate or with inhibitors of microsomal triglyceride transfer protein to promote lipid accumulation in the cultured HepaRG cells. This will allow us to investigate whether farnesol could be effective for treating pathophysiological abnormalities such as hepatic steatosis.

**INTRODUCTION**

Non-alcoholic fatty liver disease (NAFLD), which is characterized by the accumulation of lipids in liver, is prevalent in almost 30% of the world’s adult population and in 60-80% of diabetic and obese patients [1]. An association between NAFLD and cardiovascular disease (CVD) is becoming increasingly recognized, and several studies suggest that NAFLD is an independent risk factor for CVD. Thus, pharmacological interventions that could prevent or reverse hepatic steatosis could potentially decrease the overall risk of CVD in NAFLD patients.

Farnesol is an isoprenoid that is produced endogenously from farnesyl pyrophosphate, an intermediate metabolite in the cholesterol biosynthesis pathway [2], or that can be acquired through dietary intake of plant products such as tomatoes, peaches, and strawberries [3]. Both in vivo and in vitro studies have suggested that farnesol and its metabolites can modulate various physiological processes including lipid metabolism. For example, in rodents, farnesol treatment lowered serum triglycerides (TG) in diabetic and obese patients [1]. An association between NAFLD and cardiovascular disease [4]. Both farnesol and its metabolites can modulate lipid metabolism using a cell culture model of human steatosis.

**METHODS**

HepaRG cell culture and differentiation: HepaRG cells were obtained from Bioresource International under a Material Transfer Agreement with RIBIT-Transfert (Fons, France). The cells were cultured in HepaRG growth medium (Williams’ Medium E supplemented with 10% FBS, ScienCell Research Laboratories, CA). HepaRG cells were then differentiated into a hepatocyte-like cell line by culturing them for 3 to 4 days in media containing 2% FBS and 100 U/ml of ascorbic acid and 0.1M triamcinolone acetonide (Williams’ Medium E supplemented with 10% FBS, 5µg/ml insulin, 0.1M triamcinolone acetonide, and 100U/ml of ascorbic acid. ScienCell Research Laboratories (Carlsbad, CA). TG content was normalized to cellular protein levels.

**RESULTS**

Figure 2A: Effect of dietary intake of plant products such as tomatoes, peaches, and strawberries on lipid metabolism in HepaRG cells. Differentiated HepaRG cells were treated with plant products (100µg/ml) for 72 hours. Cells were then harvested for measurement of TG levels.

**CONCLUSIONS**

Treatment of HepaRG cells with prototypical activators for CAR (CITCO), FXR (GW6496 and COCA), or PPARα (GW15164) increased expression of respective target genes, demonstrating that the CAR, FXR, and PPARα pathways are intact in HepaRG cells. Treatment with farnesol increased CYP2B6 mRNA levels, most likely through the activation of CAR. However, farnesol treatment did not increase SHP or PLIN2 expression, suggesting that farnesol has little effect on FXR or PPARα activity in HepaRG cells under standard culture conditions.

Treatment with oleic acid produced a concentration-dependent increase in intracellular lipid levels, as observed by increased optical density in red O staining.

Oleic acid-treated HepaRG cells also had higher intracellular TG levels compared to controls. Co-treatment with farnesol lowered the OA-induced TG levels by ~25%. This suggests that farnesol may suppress OA-induced increases in intracellular TG levels.

Treatment with oleic acid changed the expression of genes involved in hepatic fatty acid β-oxidation. CYP1A2 expression was decreased by OA treatment, and farnesol treatment restored the mRNA level back to control. Co-treatment with farnesol lowered the OA-induced TG levels by ~25%. This suggests that farnesol may suppress OA-induced increases in intracellular TG levels.

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