224

Inhibition of AMPK-HMGCR signaling promotes the development of atherosclerosis and hepatic steatosis

Kim Loh [1,*], Shanna Tam [1], Gregory Steinberg [2], Morgan Fullerton [3], Sandra Galic [1], Bruce Kemp [1]

[1] *St. Vincent's Institute of Medical Research, Fitzroy, VIC, Australia*
[2] *Biochemistry and Biomedical Sciences, McMaster University, Hamilton, ON L8N 3Z5, Canada*
[3] *Biochemistry, Microbiology and Immunology, University of Ottawa, Ottawa, ON K1H 8M5, Canada*

AMP-activated kinase (AMPK) regulates multiple signaling pathways involved in glucose and lipid metabolism in response to changes in hormonal and nutrient status. Cell culture studies have shown that AMPK phosphorylation and inhibition of the rate-limiting enzyme in the mevalonate pathway–HMG-CoA-Reductase (HMGCR) at Ser871 (human HMGCR Ser872) suppresses cholesterol synthesis. In order to evaluate the role of AMPK-HMGCR signaling *in vivo* we generated mice with a Ser871Ala knock-in mutation (HMGCR KI). Cholesterol synthesis was significantly suppressed in WT but not in HMGCR KI hepatocytes in response to AMPK activators. Consistently, liver cholesterol synthesis and cholesterol levels were significantly up-regulated in HMGCR KI mice, resulting in increased plaque size when crossed onto atherosclerosis-prone background. When fed a high carbohydrate diet HMGCR KI mice had enhanced triglyceride synthesis and liver steatosis resulting in impaired glucose homeostasis. Our results demonstrate that AMPK-HMGCR signaling alone is sufficient to regulate both cholesterol and triglyceride synthesis under conditions of a high carbohydrate diet. These findings highlight the tight coupling between the mevalonate and fatty acid synthesis pathways as well as revealing a role of AMPK in suppressing the deleterious effects of a high carbohydrate diet.

https://doi.org/10.1016/j.orcp.2018.11.147

225

Potential actions of a Chinese herbal formula for obesity

Shiqi Luo [1,*], George Lenon [1], Harsharn Gill [2], Daniel Dias [1], Andrew Hung [2]

[1] *School of Health and Biomedical Sciences, RMIT University, Bundoora, VIC, Australia*
[2] *School of Science, RMIT University, Bundoora, VIC, Australia*

Obesity has become a world-wide health concern. Natural products and Chinese herbs are popular alternatives for weight reduction however the mechanism of actions remains unclear. Slimming plus (SP) formula is a Chinese herbal formula modified from RCM104, which was previously proven to be effective for weight reduction in the clinical trial by our research team. The aim for this study is to investigate the mechanisms of the SP formula and provide scientific rationale for its effectiveness.

Methods: 73 compounds/ligands and 2 targeted proteins (pancreatic lipase and amylase) were chosen from literature review. Autodock software Pyrx was used for virtual molecular screening, which can predict the interactions between the ligands and targeted proteins. In addition, SP formula was qualitatively analysed via high-performance thin-layer chromatography (HPTLC) against 10 bioactive chemical references that are known for weight-loss.

Results: Molecular docking showed that the main chemical components from each herb of SP formula have higher binding affinities (kcal/mol) compared to the control Orlistat and Acarbose (lipase and amylase inhibitor respectively). The leading compounds for the target proteins have been predicted as chrysophanol, caffeine, genisterin, gallic acid, genipin, alismol, catapol, eburicoic acid according to their efficiency scores, which are almost twice as high as that of the control. HPTLC results indicated that the SP formula contains bioactive compounds for weight reduction such as epigallocatechin gallate (EGCG), caffeine etc.

Conclusion: Molecular docking and HPTLC have preliminarily explored the potential mechanisms of the SP formula for weight reduction. Further studies such as inhibition assays will be followed for verifying the lipase and amylase inhibitory activities of the SP formula.

https://doi.org/10.1016/j.orcp.2018.11.148

226

Inhibition of palmitic acid-induced hepatic steatosis and inflammation with Tempol

Gihani Manodara [1,*], Alison K. Heather [2], Kristine C.-Y. McGrath [1]

[1] *School of Life Science, University of Technology, Sydney, NSW, Australia*
[2] *Department of Physiology, University of Otago, Dunedin, New Zealand*

Background and aim: Obesity is associated with an increased risk of non-alcoholic fatty liver disease (NAFLD) that characterised by the accumulation of lipids in the liver. Excessive intrahepatic lipid accumulation is associated with alteration of glucose, fatty acid metabolism and is known to drive inflammation and oxidative stress, ultimately leading to the development of hepatic and systemic insulin resistance. The aim of this study was to assess the effects of a nitroxide, Tempol, on the accumulation of lipid and inflammation induced by palmitic acid (PA) in human liver cells.

Methods and results: To assess the effects of Tempol, HepG2 cells were exposed to PA for 24 hours before exposure to Tempol (200 μM, 500 μM, 1 mM, 2 mM) for 5 hours. The effects of PA induced-cellular steatosis and inflammation were then assessed using an (i) oil red O staining and extraction method to assess lipid accumulation, (ii) RT-qPCR to assess the mRNA gene expression level of inflammatory markers, (iii) glycogen assay to assess glycogen levels (iv) dichlorofluorescein assay to assess reactive oxygen species (ROS) levels and (v) MTT assay to detect cell viability. The results show HepG2 exposed to TEMPOL reduced palmitic acid-induced- (i) lipid accumulation, (ii) inflammation, (iii) insulin-mediated glycogenesis and (iv) reactive oxygen species levels with (v) no effect on cell viability.

Conclusions: These findings suggest that Tempol plays a protective role in PA-induced hepatic steatosis that is associated with reduced hepatic inflammation, oxidative stress and insulin-mediated glycogenesis, thus indicating Tempol may be useful as a pharmaceutical therapy for improving hepatic steatosis and insulin sensitivity.

https://doi.org/10.1016/j.orcp.2018.11.149