AMPK-mediated HMGCR regulation of cholesterol metabolism in macrophages

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Abstract

Atherosclerosis, the accumulation of cholesterol-loaded macrophages (foam cells) in the arteries, leads to cardiovascular disease, which is the leading cause of mortality in the developed world. 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) is the rate-limiting step in cholesterol biosynthesis and is regulated by several mechanisms, including inhibition by reversible phosphorylation by the energy-sensing AMP-activated protein kinase (AMPK). AMPK activators are under investigation for their potential as anti-atherogenic agents. The purpose of this paper was to test the physiological significance of AMPK regulating HMGCR activity in macrophages. Using bone marrow-derived macrophages from HMGCR knock-in (KI) mice, where the phosphorylation site had been eliminated, this paper investigated the impact that AMPK signaling to HMGCR has on cholesterol synthesis, accumulation of cholesterol, and cholesterol efflux. Since atherosclerosis is tightly linked with inflammation in plaques, cytokine expression was also assessed after treating macrophages with a pro-inflammatory stimulus. While there was increased cholesterol synthesis in macrophages from HMGCR KI mice than wild-type (WT) mice, there were no differences between WT and HMGCR KI macrophages in all the other aspects examined. This suggests that the regulation of HMGCR through phosphorylation by AMPK is not physiologically significant to the development of atherosclerosis. Further investigation is required in vivo to confirm this finding. To further understand how AMPK activators may be a potential drug strategy for treating atherosclerosis, other pathways and mechanisms need to be investigated.

Methods

- **Macrophage culture**: Bone marrow was isolated from the tibia and femur, and left to differentiate into macrophages in the presence of L929 (a source of macrophage colony stimulating factor) for 7 days. Cells were then scraped, counted and seeded for subsequent experiments.
- **Lipogenesis assay**: BMDMs from WT and HMGCR KI mice were treated with 1 μg/mL [3H] sodium acetate for 4 hours. Lipids were extracted using the Bligh and Dyer method. Radioactivity in lipids was determined by liquid scintillation counting (LSC). Cholesterol was separated from total lipids using thin-layer chromatography. Radioactivity in cholesterol was determined by LSC.
- **Cholesterol efflux**: BMDMs were treated with 0.5 μg/mL [3H] cholesterol for 30 hours, then with either A-769662 (100 μM), T0901317 (100 μM) or DMSO for 24 hours, and then with cholesterol acceptor ApoA1 (5 μg/mL) or HDL (50 μg/mL) for a final 24 hours. The radioactivity in the media and in the cells were determined by LSC.
- **Cholesterol accumulation**: BMDMs were incubated in acetylated LDL (50 μg/mL) for 30 hours, with and without A-769662 (100 μM). Cholesterol was quantified using the Amplex Red Cholesterol Assay Kit.
- **Proinflammatory cytokine expression**: BMDMs were incubated with lipopolysaccharide (LPS: 100 ng/mL), with and without A-769662 (100 μM), for 6 hours. After RNA isolation and cDNA synthesis, quantitative PCR was performed to determine the expression of TNF-α and IL-6. ELISAs were performed to quantify cytokine secretion.

Results

- **Cholesterol efflux to ApoA1 and HDL**: T0901317 increased cholesterol efflux to apoA1 and HDL compared to vehicle (Figure 3). A-769662 increased cholesterol efflux to apoA1 and HDL compared to vehicle (Figure 3).
- **Gene expression of proinflammatory cytokines**: T0901317 increased TNF-α expression compared to vehicle (Figure 4). A-769662 increased TNF-α expression compared to vehicle (Figure 4).

Conclusions

- **Cholesterol synthesis was higher in macrophages derived from HMGCR KI mice than WT mice when AMPK was activated**.
- There were no significant differences between the between the WT and HMGCR KI macrophages in terms of total lipid synthesis, cholesterol accumulation, cholesterol efflux or proinflammatory cytokine expression.
- Therefore, the phosphorylation of HMGCR by AMPK is not physiologically significant to the development of atherosclerosis.
- Future work could examine this regulation in vivo.

References