The Action of Phytochemicals on Lipid Accumulation and Lipotoxicity in HepG2 Cultured Cells

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INTRODUCTION

- Non-alcoholic fatty liver disease (NAFLD) is becoming more prevalent in elderly populations, due to the increasing incidence of risk factors – unhealthy and unbalanced diets.
- The ‘two-hit’/’multi-hit’ hypothesis has been proposed in the spectrum of NAFLD (Ludwig et al., 1997, James and Day, 1998). This states that steatosis, which is lipid accumulation in the liver, is an established ‘first hit’ which is required to increase susceptibility of hepatocytes to further ‘second hits’ which then contribute to more severe NAFLD pathologies including non-alcoholic steatohepatitis (NASH).
- A key ‘second hit’ is fatty acid-mediated oxidative stress causing lipotoxicity and eventually NASH (see diagram).
- Treatment options are currently limited. Due to the presence of lipid accumulation in developing steatosis and oxidative stress in progressing to NASH, anti-steatotic or antioxidant compounds could offer preventative/treatment measures.
- Therefore, the aim of this experiment was to determine possible protective actions of 4 phytochemicals, (quercetin dihydrate, (-)-epigallocatechin gallate, L-sulforaphane and indole-3-carbinol) with established anti-steatotic and antioxidant properties, against lipid accumulation or oxidative stress, as potential novel NAFLD therapy.

METHOD

- Oleate and palmitate are endogenous FAs.
  - Oleate causes lipid accumulation which could develop into steatosis
  - Palmitate causes lipid-induced oxidative stress and lipotoxicity leading to NASH
- HepG2 cells, from a human well-differentiated hepatoblastoma cell line, were exposed to either oleate (providing a model for steatosis) or palmitate (providing a model for NASH) alongside one of the phytochemicals for 24 hours.
- We also used control runs in which the cells were exposed to only the phytochemical and no fatty acid to identify the effect of the phytochemical in the absence of the damage-inducing fatty acid.
- After 24 hours, Nile Red was used to measure changes in lipid accumulation and Neutral Red measured changes in viability, caused by the phytochemicals.

CONCLUSIONS AND FUTURE DIRECTIONS

- To conclude, statistical significance for the effects of oleate and palmitate highlighted that the model works and could be used in future experiments.
- However, none of the phytochemicals exhibited a significant protective effect against oleate-mediated lipid accumulation or palmitate-mediated lipotoxicity and therefore could not be suggested to protect against steatosis or lipid-induced oxidative stress in the development of NASH. Therefore, there is insufficient evidence to suggest that these 4 phytochemicals have potential ability to prevent or limit the development of NASH.
- Further research is therefore necessary and ideas for future experiments would include:
  - Using alternative phytochemicals to investigate their effects
  - Altering chemical exposure sequence - Adding palmitate/oleate at a different time to the phytochemical and not together may enhance the effect of either on the cells leading to different results
  - Using a shorter time exposure to the fatty acids/phytochemicals
  - Using models with cells exposed to combinations of different ratios of oleate/palmitate which would be a more realistic representation of in vivo situations

REFERENCES

