Concurrent Session 8A: PUFA/Heart Disease

Influence of catechin and epicatechin on oleic acid fat loaded HepG2 cells

M Janevski[1], J Antonas[2], P Lewandowski[1]
[1]*School of Medicine, Deakin University, Vic 3216*
[2]*School of Biomedical and Clinical Sciences, Victoria University, Vic 3021*

Background – Non-alcoholic steatohepatitis (NASH) is a condition in which hepatocytes become fat loaded and further damaged by oxidative stress.
Objectives – To induce oxidative stress by fat loading HepG2 cells and investigate the ability of catechin/epicatechin to counter this effect. To investigate the influence of these conditions on the expression of key NADPH oxidase (NOX) genes responsible for the production of reactive oxygen species (ROS).
Design – HepG2 cells were exposed to one of three conditions: control media, control media with 0.5mM oleic acid (48hrs), or control media with oleic acid (48hrs) followed by a catechin (0.16µmol/L) epicatechin (5.92µmol/L) combined treatment (2hrs). Triglyceride concentration was measured and ROS detected using a dihydroethidium stain (DHE). Relative gene expression was analysed by real time PCR for NOX1, NOX3, NOX4 and $p47^{phox}$.
Outcomes – Fat loading HepG2 cells increased ROS by 90% compared to control and increased triglyceride concentration 1.5 fold compared to control ($p=0.01$). Treatment with catechin/epicatechin decreased ROS by 60% compared to fat loaded cells. Triglyceride concentration decreased with treatment by 2.7 fold compared to control ($p<0.01$) and a 4 fold decrease when compared to fat loaded cells ($p<0.01$). Interestingly only NOX1 relative gene expression increased in catechin/epicatechin treated groups compared to control and fat loaded cells ($p<0.05$).
Conclusion – Fat loading HepG2 cells increases intracellular triglycerides, a common finding in NASH, and also increases ROS. Despite lower levels of ROS in catechin/epicatechin treated cells, relative expression of NOX1 was increased, indicating that NOX1 may act as a signalling pathway to reduce ROS in liver disease.

Pure dietary flavonoids, quercetin and (-)-epicatechin augment nitric oxide products and reduce endothelin-1 acutely in healthy men

WM Loke[1,2], JM Hodgson[1], JM Proudfoot[1], AJ McKinley[2], IB Puddey[1], KD Croft[1]
[1] *School of Medicine and Pharmacology,* [2] *School of Biomedical, Biomolecular and Chemical Sciences, University of Western Australia, Perth, Western Australia*

Background – Dietary flavonoids may reduce cardiovascular disease risk by improving endothelial function.
Objective – To investigate whether pure dietary flavonoids may improve endothelial function by modulating nitric oxide (vasodilator), endothelin-1 (vasoconstrictor) and F_2-isoprostane (marker of oxidative stress) production.
Design – A randomised, placebo controlled, cross-over trial in 12 healthy men was conducted to compare the acute effects on nitric oxide, endothelin-1 and F_2-isoprostanes after oral administration of 200 mg of quercetin, (-)-epicatechin and epigallocatechin gallate. A blood and spot urine sample were taken at baseline, a blood sample was taken at 2 h post treatment, and a 5 h urine collection was performed. Measurements performed in plasma and urine included: *S*-nitrosothiols (plasma only), nitrite and nitrate concentrations (markers of nitric oxide production); endothelin-1; F_2-isoprostanes, and quercetin, (-)-epicatechin and epigallocatechin gallate.
Outcomes – Relative to water (control), quercetin and (-)-epicatechin resulted in a significant increase in plasma *S*-nitrosothiols, plasma nitrite, and urinary nitrate concentrations ($p < 0.05$ for all), but not plasma nitrate or urinary nitrite. Epigallocatechin gallate did not alter any measures of nitric oxide production. Quercetin and (-)-epicatechin, but not epigallocatechin gallate, resulted in a significant reduction in plasma endothelin-1 concentration ($p < 0.05$), and only quercetin significantly decreased urinary endothelin-1 concentration ($p < 0.05$). None of the treatments significantly altered plasma or urinary F_2-isoprostane concentrations. Significant increases in the circulating concentrations of the three flavonoids were observed after the corresponding treatment ($p < 0.05$).
Conclusion – Dietary flavonoids such as quercetin and (-)-epicatechin, but not epigallocatechin gallate, can augment nitric oxide status and reduce endothelin-1concentrations. These may be important pathways to improved endothelial function. We have also found that the flavonoids tested had no acute effect on systemic oxidative stress assessed using F_2-isoprostanes.