



Novel raspberry triterpenoids with potential anti-cancer effects

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The anticancer properties of bioactive phytochemicals within berries are of interest given the inverse correlation of fruit and vegetable consumption with the incidence of colorectal cancer (CRC)¹. Berries are one of the most commonly consumed sources of polyphenols and these compounds may exert protective effects against initiation of CRC by reducing DNA damage². The aim of this study was to assess the bioactivity of raspberry phytochemicals identified *in vivo* with respect to markers of gut health.

Using non-targeted LC-MSⁿ of ileal fluids from eleven ileostomates who completed a raspberry feeding study³, we identified novel compounds and putatively identified major components as fruit triterpenoid glycosides. The major triterpenoid had an apparent MW of 680 and could be purified from raspberry seeds. The anti-genotoxic activity of this triterpenoid-enriched fraction (TRF) was assessed by COMET assay using HT29 (adenocarcinoma) and CCD841 CoN (normal epithelial) cells. For the *in vitro* studies both cell lines were incubated for 24 h with 100 nM of either the raspberry TRF or the positive control, a pure synthetic triterpenoid 2-cyano-3,12-dioxoolean-1,9-dien-28-oic acid (CDDO) and then challenged with 75 μM hydrogen peroxide. Further, we determined if the treatments modulated gene expression of the Nrf2-ARE pathway involved in oxidative stress cytoprotection⁴, namely nuclear factor (erythroid-derived 2)-like 2 (Nrf2), NAD(P)H Dehydrogenase, Quinone-1 (NQO1) and Heme oxygenase-1 (HO-1)⁴. Experiments were carried out as independent triplicates. ANOVA was applied to test for significant differences between means compared to untreated control using Dunnett T test (post hoc).

Treatment of HT29 cells with 100 nM of with either TRF or CDDO significantly decreased DNA damage by ~40–45 % in response to oxidative challenge ($p < 0.01$). In the normal CCD841 CoN cells, DNA damage was also reduced by ~50–55% for both treatments ($p < 0.01$). Efficacy between cell lines was not significantly different. With respect to Nrf2-ARE pathway in HT29 & CCD841 CoN cells, CDDO treatment significantly increased expression ($p < 0.05$) of all the target genes (Nrf2, NQO-1 and HO-1). TRF treatment of HT29 cells significantly increased Nrf2 expression ($p < 0.05$), but it resulted in a decreased expression of NQO1 and HO-1 ($p < 0.05$). In CCD841 CoN cells, TRF treatment increased expression of Nrf2 and NQ1 but significantly reduced the expression of HO-1 ($p < 0.05$).

To conclude, we identified a novel raspberry triterpenoid (TRF) from ileal fluid following raspberry consumption; therefore these components survive intestinal digestion and would likely enter the colon *in vivo*. We have subsequently demonstrated that the colon-available TRF can reduce DNA damage in normal colonocytes, mediated in part by the Nrf2 pathway, at a physiologically relevant concentration.

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4. Saw C.L., Kong AN (2011) *Expert Opin Ther Targets*. **15**, 281–95.