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OLEUROPEIN EXERTS ANTIOXIDANT ACTIVITY AND REDUCES THE EXPRESSION OF COX-2 AND PROINFLAMMATORY CYTOKINES IN CACO-2 CELLS AND COLONIC MUCOSA FROM ULCERATIVE COLITIS PATIENTS

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BACKGROUND AND AIM

Oleuropein (OLE) is the major secoiridoid of olive tree leaves and its antioxidant and anti-inflammatory activities have been demonstrated *in vitro* and in *in vivo* animal models. The aim of this study was to investigate the activity of OLE in the colonic mucosa from patients with ulcerative colitis (UC).

MATERIALS AND METHODS

First, the toxicity, proliferative and antioxidant effects of OLE were assessed in human epithelial colorectal adenocarcinoma (Caco-2) cells using the MTT assay and ELISA (xMark™ Microplate Absorbance Spectrophotometer, Bio-Rad). Cells were seeded at a density of $15x10^3$ cells/0.3 cm^2 in 96-wells plates, treated with different concentrations of OLE (10, 25, 50, 100 e 150 µM) for 3 h and then with hydrogen peroxide (H_2O_2 300 µM). Untreated cells and cells treated with only H_2O_2 300 µM for 1 h were used as negative and positive controls, respectively. Second, biopsies obtained during colonoscopy from 14 patients with active UC (8 M, age range 39-80 years, median 59; Mayo score 4-9, median 6) were immediately placed in an organ culture chamber and challenged with or without lipopolysaccaride from Escherichia coli (EC-LPS) at 1 µg/mL in the presence or absence of OLE 3mM for 24 h. Preliminary experiments determined the dose range and time course for incubation with OLE. Levels of cyclooxygenase (COX)-2 (COX-2/GAPDH ratio) and IL-6, IL-8, MCP-1, VEGF, TNFα, IL-1α, IL-1β cytokines (ng/ml) were assessed in total protein extracts from treated colonic biopsies and culture supernatant by Western blotting and Biochip Array on Randox Evidence Investigator (Randox Laboratories), respectively.

RESULTS

Treatment with OLE did not show toxicity on Caco-2 cells while significantly improving cell viability when compared with untreated cells (i.e., 121±16 % at 50 µM) or cells treated with only H_2O_2 (i.e., 67±3 % vs 53±6 % at 100 µM). In colonic mucosal biopsies from UC patients, levels of COX-2 were significantly lower in samples treated with OLE when compared with untreated (0.67±0.16 a.u. *vs* 0.84±0.16 a.u., p = 0.03) as well as in samples treated with OLE+EC-LPS when compared with those treated with EC-LPS alone (0.80±0.15 a.u. *vs* 1.06±0.19 a.u., p = 0.003). Accordingly, the level of each of the cytokines IL-6, IL-8, MCP-1, VEGF, TNFα, IL-1α, and IL-1β was significantly lower (p = 0.02 to 0.003) in culture supernatants of colonic samples treated with OLE or OLE+EC-LPS compared with untreated samples or samples treated with EC-LPS alone, respectively.

CONCLUSION

Non-toxic, proliferative and antioxidant activities of OLE have been demonstrated in epithelial colonic cells. Together with the anti-inflammatory effects exhibited in human *ex-vivo* experiments, this suggests the possible use of OLE for treatment of UC patients.

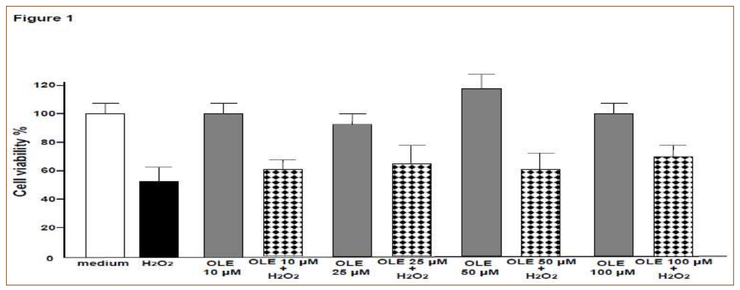

Figure 1.
Proliferative and antioxidant effects of oleuropein (OLE) in CaCo-2 cells, as assessed by cell viability (%) (MTT assay and ELISA). Cells were treated with different concentration of OLE for 3 h and then with H_2O_2. Untreated (medium) cells and cells treated with only with H_2O_2 for 1 h stand for negative and positive controls, respectively.

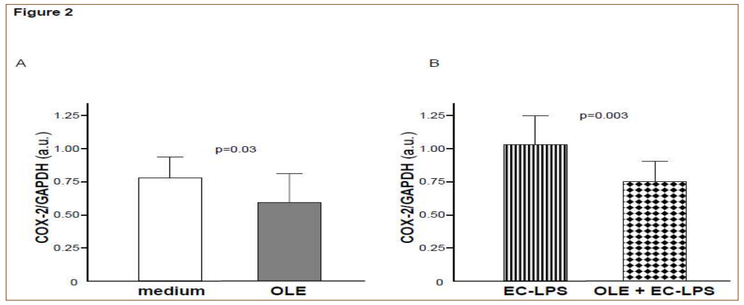

Figure 2.
Levels of cyclooxigenase (COX)-2 by Western blotting in total protein extracts from colonic biopsies of patients with ulcerative colitis treated with oleuropein (OLE) 3 mM or medium (A) and with lipopolysaccaride from Escherichia coli (EC-LPS) at 1 µg/mL or OLE+EC-LPS (B) for 24 h in an organ culture chamber. GAPDH was used as loading control. Values are expressed as mean values ± SD of arbitrary units (a.u.).

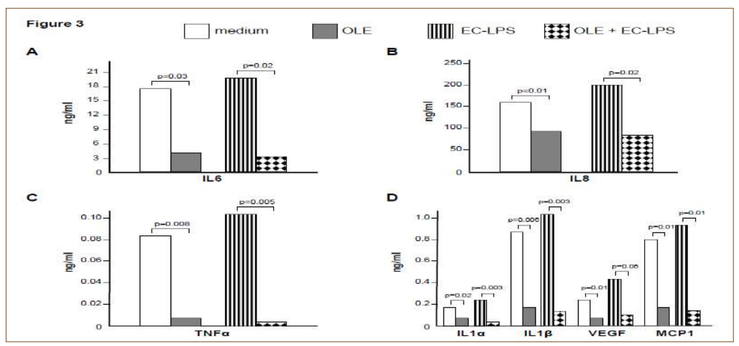

Figure 3.
Levels of pro-inflammatory cytokines by Biochip Array on Randox Evidence Investigator (Randox Laboratories) in culture supernatants of colonic biopsies of patients with ulcerative colitis treated with oleuropein (OLE) 3 mM or medium and with lipopolysaccaride from Escherichia coli (EC-LPS) at 1 µg/mL or OLE+EC-LPS for 24 h in an organ culture chamber. Data are given as ng/ml mean values.