**In vitro** approach to evaluate the role of gut phenolic metabolites in the modulation of inflammation and atherosclerosis

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**REFERENCES**

In conclusion, these preliminary results seem to support the capacity of gut phenolic metabolites to modulate the endothelial cell adhesion process. The reduction in the adhesion process seems to be driven by a decrease of E-selectin levels but not VCAM-1. Ongoing experiments are attempting to clarify the mechanisms of action of each compound involved in the above observations.

**BACKGROUND:** Inflammation is a common process in endothelial dysfunction, characterized by an increase in the expression of endothelial cell adhesion molecules and a decrease of nitric oxide production [1]. Evidence suggests that polyphenols may play a beneficial role in attenuating inflammation with implications for atherosclerosis [2]. However, their mechanism of action is still not entirely understood due to their poor absorption and extensive intestinal and hepatic transformation [3].

**OBJECTIVE:** This study aims to evaluate some of gut phenolic metabolites (i.e. protocatechuic, gallic, syringic and vanillic acid; PA, GA, SA, VA, respectively) to reduce the adhesion of monocytes (THP-1) to endothelial cells (HUVECs), in a stimulated pro-inflammatory environment, and to decrease the production of cell adhesion molecules (VCAM-1 and E-selectin), as potential markers involved in such modulation.

**METHODS:**

**Gut phenolic metabolites tested**

- Protocatechuic acid (PA)
- Gallic acid (GA)
- Vanillic acid (VA)
- Syringic acid (SA)

**Cell viability evaluation**

- **Cell viability assessed by trypan blue assay**
  The viability assay was carried out for each compound (PA, GA, VA and SA) and for each concentration (from 0.01 to 10µg mL⁻¹). HUVEC were incubated for 24 h with the compounds and successively, trypan blue assay was performed in triplicate.

- **Cell viability assessed by MTT assay**
  The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium reduction assay was performed on HUVECs treated with the maximum concentration for gut phenolic metabolites. The assay was performed in triplicate following the instructions reported in the kit.

**RESULTS:**

- GA and PA significantly decreased the adhesion of THP-1 to HUVECs at 1 µg mL⁻¹ (-18.0%; p=0.04 for GA, -29.5%; p=0.03 for PA) and 10 µg mL⁻¹ (-59.3%; p<0.001 for GA, -44.3%; p=0.001 for PrA) compared to TNF-α. VA significantly reduced the adhesion only at the maximum concentration (-20.8%; p<0.005). No effect was observed after SA supplementation.

- GA and PA significantly decreased the production of E-selectin at 1 µg mL⁻¹ (-76.6%; p<0.001 for GA, -72.0%; p<0.001 for PA) and 10 µg mL⁻¹ (-77.7%; p<0.001 for GA, -73.8%; p<0.001 for PA) compared to TNF-α. VA significantly reduced the production of E-selectin only at the maximum concentration (-65.1%; p<0.005). No effect was detected following SA supplementation.

**Adhesion of THP-1 to HUVECs**

- Day 1-Preparation of 96 wells plate (2x10⁴ HUVEC per well);
- Day 2-Labeling of THP-1 cells with CellTrackerTM Green CMFDA, addition of THP-1 (2x10⁴ cells/well) and TNF-α (100 ng mL⁻¹) to HUVEC, and incubation for 24h;
- Day 3-Incubation with PA, GA, VA and SA at different concentrations (from 0.01 till 10µg mL⁻¹) for 24 h;
- Day 4- Reading of the fluorescence (excitation: 485 nm, emission: 538 nm, mod. F200 Infinite, TECAN Milan, Italy)

**Evaluation of cell adhesion molecules**

- The levels of vascular cell adhesion molecules (VCAM-1), and endothelial selectin (E-selectin) were measured in the supernatant by ELISA kits following the manufacturer’s instruction.
- The results derived from three independent experiments.

**CONCLUSION:**

- GA and PA significantly decreased the production of E-selectin at 1 µg mL⁻¹ (-76.6%; p<0.001 for GA, -72.0%; p<0.001 for PA) and 10 µg mL⁻¹ (-77.7%; p<0.001 for GA, -73.8%; p<0.001 for PA) compared to TNF-α. VA significantly reduced the production of E-selectin only at the maximum concentration (-65.1%; p<0.005). No effect was detected following SA supplementation.

- No significant effect was observed on the levels of VCAM-1.