ROLE OF PHENOLIC ACIDS IN THE MODULATION OF MONOCYTES ADHESION TO ENDOTHELIAL CELLS AND VASCULAR ADHESION MOLECULES IN A TNF-α STIMULATED PRO-INFLAMMATORY ENVIRONMENT

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BACKGROUND

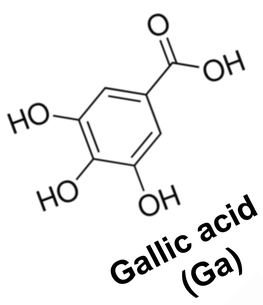
Inflammation and oxidative stress play an important role in the early step of atherosclerosis through the activation of endothelial cells (ECs) and the expression of cytokines and adhesion molecules (i.e. intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), E- and P-selectins) involved in the recruitment, rolling and adhesion of monocytes to ECs.[1-2]

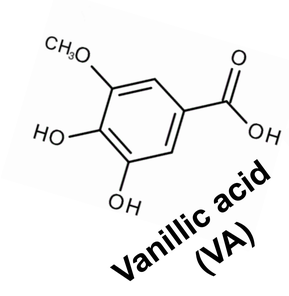
Polyphenols, including phenolic acids, flavonoids, stilbenes and lignans, have been proposed to exert numerous biological activities including a promising role in counteracting atherosclerosis.[3] However, very little is known about the mechanisms by which polyphenols exert their biological activity. Basing on these premises, there still is the need for researches focused on the comprehension of the potential mechanisms of these bioactive compounds in preventing atherosclerosis ~~formation~~.

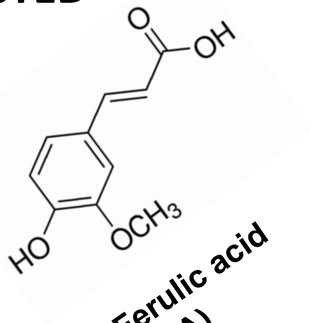
OBJECTIVE

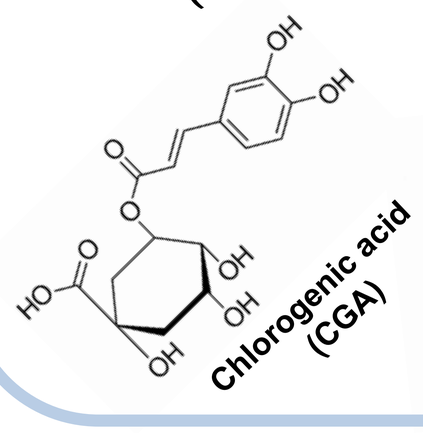
The objective of the present study is to evaluate the capacity of several phenolic acids such as, gallic, vanillic, chlorogenic, caffeic, ferulic and hippuric acid, to couteract the adhesion of monocytes to stimulated endothelial cells as early event in atherosclerosis. In addition, the production of cell adhesion molecules (VACM-1 and E-selectin) is evaluated.

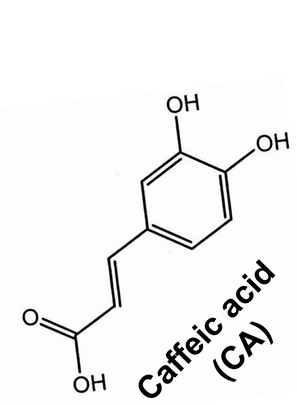
PHENOLIC COMPOUNDS TESTED

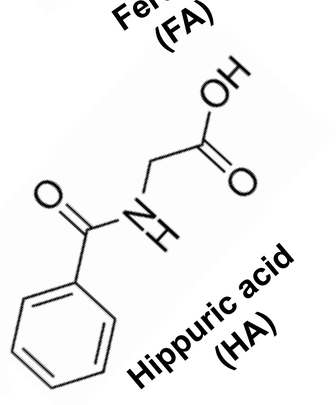












METHODS

Study of the anti-atherosclerotic effect of phenolic acids in a model of HUVECs and THP-1

- Day 1 - Preparation of 96 wells plate (2 x 10^4 HUVEC cells/well);
- Day 2 - Labelling of THP-1 cells with CellTrackerTM Green CMFDA, addition of THP-1 (2 x 10^4 cells/well) and TNF-α (100 ng mL^{-1}) to HUVEC cells, and incubation for 24 h;
- Day 3 - Incubation with phenolic acids at different concentrations (from 0.01 till 10 µg mL^{-1});
- Day 4 - Reading the fluorescence (excitation: 485 nm, emission: 538 nm). using a plate reading spectrophotometer (mod. F200 Infinite, TECAN Milan, Italy).

Evaluation of sVCAM-1 and E-selectin in the cell supernatant

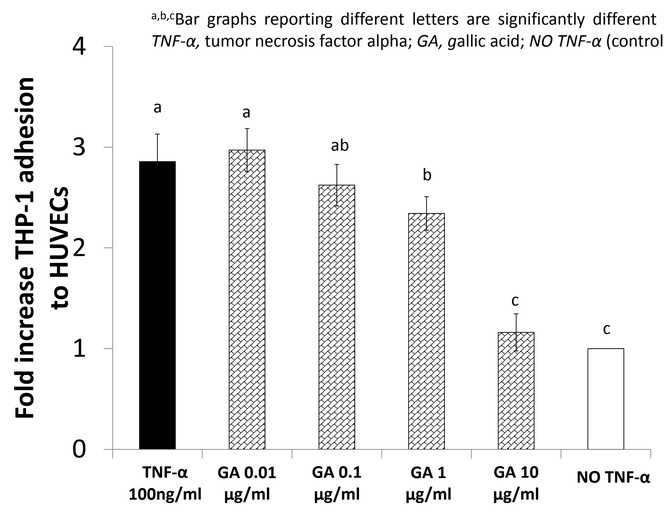
The quantification of sVCAM-1 and E-selectin was performed by ELISA kits. The absorbance was measured at 450 nm using a plate reading spectrophotometer (mod. F200 Infinite, TECAN Milan, Italy).

RESULTS

Fig.1 Effect of gallic acid (*GA 0.3–58.8 µM*) on THP-1 adhesion to HUVECs. Results are expressed as mean ± standard error of mean.

Fig.1

[a,b,c]Bar graphs reporting different letters are significantly different ($p \leq 0.05$). *TNF-α,* tumor necrosis factor alpha; *GA, g*allic acid; *NO TNF-α* (control).

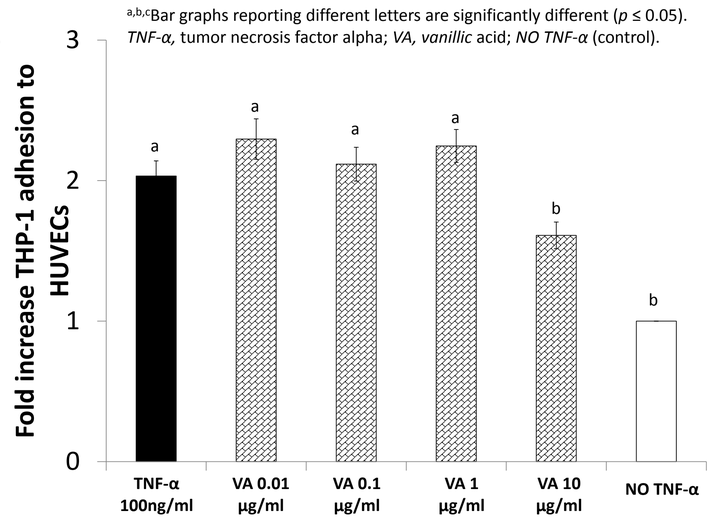


✓ GA reduces THP-1 adhesion to HUVECs at the concentration of 1µg/ml (-23.6%, p<0.001) and 10µg/ml (-27.8%; p<0.001)

Fig.2 Effect of vanillic acid (*VA 0.06-59.5 µM*) on THP-1 adhesion to HUVECs. Results are expressed as mean ± standard error of mean.

Fig.2

[a,b,c]Bar graphs reporting different letters are significantly different ($p \leq 0.05$). *TNF-α,* tumor necrosis factor alpha; *VA, vanillic* acid; *NO TNF-α* (control).



✓ VA reduces THP-1 adhesion to HUVECs at the maximum concentration (10µg/ml; -20.8%; p<0.005)

Table 1-Effect of GA and VA on the levels of E-selectin and VCAM-1 in the cell supernatant. Results are expressed as mean ± standard error of mean.

Data derived from three different experiments and each concentration tested in triplicate. *GA,* gallic acid; VA, vanillic acid; *TNF-α,* tumor necrosis factor alpha. Concentration range: 0.3–58.8 µM for GA and 0.06-59.5 µM for VA. [a,b,c]Data with different letters are significantly different (p <0.05).

	E-SELECTIN (pg/ml)		VCAM-1 (ng/ml)	
Concentrations	GA	VA	GA	VA
0.01 µg mL^{-1}	304±15[a]	321±29[a]	17±1.8	14±0.4
0.1 µg mL^{-1}	321±11[a]	312±46[a]	15±1.9	14±1.7
1 µg mL^{-1}	206±10[b]	306±14[a]	16±0.7	14±1.1
10 µg mL^{-1}	188±17[b]	110±43[b]	16±1.8	14±4.1
(TNF-α) 100 ng mL^{-1}	318±12[a]	316±16a	17±1.8	14±3.6
(TNF-α) 0 ng mL^{-1}	65±4.6c	60.7±17c	11±0.3	7.2±2.8

✓ GA reduces E-selectin levels at the dose of 1µg/ml (-34%, p<0.01) and 10µg/ml (-40%; p<0.01), while VA only at the maximum concentration (10µg/ml; -65%; p<0.01).

✓ No effect on VCAM-1 levels was observed

- Preliminary experiments have shown no effect following caffeic and chlorogenic acid supplementation
- The role of ferulic and hippuric acid has not yet been evaluated

CONCLUSION

The preliminary results seem to support the capacity of some phenolic acids to counteract THP-1 adhesion to HUVECs and to reduce the production of E-selectin. The effects seem to be compound and dose dependent. In particular, gallic and vanillic acid have shown to reduce the adhesion of monocytes to activated endothelial cells but only at the high doses. Conversely, preliminary data on caffeic and chlorogenic acid did not show any significant effect. Further experiments are ongoing in order to better clarify the specific activity of each compound and the mechaninsm of action involved.

REFERENCES

1) Gimbrone MA & García-Cardeña G. Endothelial Cell Dysfunction and the Pathobiology of Atherosclerosis, Circ Res. 2016 ; 118(4): 620–636. 2) Manach C et al., Addressing the inter-individual variation in response to consumption of plant food bioactives: Towards a better understanding of their role in healthy aging and cardiometabolic risk reduction. Mol Nutr Food Res. 2017; 61(6): 1600557. 3) Bahramsoltani et al., Dietary polyphenols for atherosclerosis: A comprehensive review and future perspectives. Crit Rev Food Sci Nutr. 2017;16:1-19.