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

Del Bo’ Cristian*, Marino Mirko, Moreletti Alessandro, Riso Patrizia, Porrini Marisa

Department of Food, Environmental and Nutritional Sciences, Division of Human Nutrition, Università degli Studi di Milano, Milan, Italy

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 counteract the adhesion of monocytes to stimulated endothelial cells as early event in atherosclerosis. In addition, the production of cell adhesion molecules (VCAM-1 and E-selectin) is evaluated.

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 10^5 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).

METHODS

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.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.

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

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

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.

<table>
<thead>
<tr>
<th>Concentrations</th>
<th>E-SELECTIN (pg/ml)</th>
<th>VCAM-1 (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GA 0.01 μg/ml</td>
<td>321±19^a</td>
<td>541.9±40^a</td>
</tr>
<tr>
<td>GA 0.1 μg/ml</td>
<td>321±19^a</td>
<td>541.9±40^a</td>
</tr>
<tr>
<td>GA 1 μg/ml</td>
<td>206±10^a</td>
<td>164.0±7^a</td>
</tr>
<tr>
<td>GA 10 μg/ml</td>
<td>188±15^a</td>
<td>164.0±7^a</td>
</tr>
<tr>
<td>VA 0.01 μg/ml</td>
<td>318±12^a</td>
<td>164.0±7^a</td>
</tr>
<tr>
<td>VA 0.1 μg/ml</td>
<td>318±12^a</td>
<td>164.0±7^a</td>
</tr>
<tr>
<td>VA 1 μg/ml</td>
<td>654±64^a</td>
<td>110.0±7^a</td>
</tr>
</tbody>
</table>

- 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

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 mechanism of action involved.

REFERENCES