# Transport and Metabolism of New Synthetic Lipophilic Derivatives, Hydroxytyrosyl Ethers, by Enterocyte-like CaCo-2 Cells

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As a response to the increasing demand by the food industry for new synthetic lipophilic antioxidants, a series of hydroxytyrosol derivatives (hydroxytyrosyl ethers[1]) have been synthesized, with similar or even higher antioxidant activity than free hydroxytyrosol[2]. These compounds provide a useful alternative to synthetic additives such as BHT that are widely used but whose use is under threat due to safety concerns, and also to natural antioxidants of which there are very few (e.g. α-tocopherol).

The transport and metabolism of alkyl hydroxytyrosyl ethers in comparison with free hydroxytyrosol was evaluated using a two-compartment transwell system containing differentiated CaCo-2 cell monolayers, which simulates the intestinal barrier. Hydroxytyrosol and a series of hydroxytyrosyl derivatives with different alkyl side chain lengths (methyl, ethyl, propyl and butyl hydroxytyrosyl ethers) were loaded apically (AP) and basolaterally (BL) in order to evaluate their apparent permeability coefficient ($P_{aap}$) and AP:BL ratio ($P_{app\ B\rightarrow A}/P_{app\ A\rightarrow B}$). In addition, LC-DAD and LC-MSn were used in conjunction with enzyme hydrolysis (sulfatase, glucuronidase) to identify the metabolites.

The $P_{app}$ ratios were high for all compounds indicating good permeability across Caco-2 monolayers. The rate of uptake and transport correlated directly with their lipophilicity. While only methylated derivatives of HTy were detected in the basolateral media, hydroxytyrosyl ethers were metabolized to glucuronidated, methylated and methylglucuronidated conjugates. However, about 50% of the compounds reaching the basolateral compartment were un-metabolised which indicates the potential for hepatic transformation.

In conclusion, alkyl hydroxytyrosyl ethers are well absorbed across, and partially metabolized by, CaCo2 cell monolayers, in keeping with their lipophilic nature.

[1] Madrona, A., Pereira-Caro, G., Mateos, R., Rodríguez, G., Trujillo, M., Fernandez-Bolaños, J., Espartero, J.L. (2009) Synthesis of hydroxytyrosyl alkyl ethers from olive oil waste waters. Molecules 14, 1762-1772.

[2] Pereira-Caro, G., Madrona, A., Bravo, L., Espartero, J.L., Alcudia, F., Cert, A., Mateos, R. (2009). Antioxidant activity evaluation of alkyl hydroxytyrosyl ethers, a new class of hydroxytyrosyl derivatives. Food Chemistry 115, 86-91.