

Metabolism of New Synthetic Lipophilic Derivatives, Hydroxytyrosyl Ethers, by Human Hepatoma HepG2 cells

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A series of hydroxytyrosol derivatives (hydroxytyrosyl ethers^[1]) with similar or even higher antioxidant activity than free hydroxytyrosol^[2], has been synthesized as a response to the increasing demand by the food industry for new synthetic lipophilic antioxidants. Their effective absorption at intestinal level was demonstrated using enterocyte like Caco-2 cells, where about 50% of the compounds reaching the basolateral compartment were un-metabolized indicating the potential for hepatic transformation. In order to evaluate the metabolism of these hydroxytyrosyl derivatives with different alkyl side chain length (methyl, ethyl, propyl and butyl hydroxytyrosyl ethers), every compound has been incubated for 2 and 18 hours with human hepatoma cells (HepG2) in culture as a model system of the human liver. LC-DAD and LC-MS were used for the identification of metabolites in culture media, cell lysates, as well as in these samples after hydrolysis with β -glucuronidase and sulfatase. In vitro conjugation reactions of pure phenols were also performed. The results showed an extensive uptake and metabolism by HepG2 cells after 18h of incubation. A direct relationship between the lipophilic nature of the compounds and the biotransformation yield was observed. Similar ratio of methylated to glucuronidated forms were detected after 2h, while at longer incubation time high amounts of methyl-glucuronidated and glucuronidated metabolites were identified, together with lower amounts of methyl conjugates. In conclusion, alkyl hydroxytyrosyl ethers are widely metabolized by the liver in keeping with their lipophilic nature.

[1]. Madrona, A., Pereira-Caro, G., Mateos, R., Rodríguez, G., Trujillo, M., Fernandez-Bolaños, J., Espartero, J.L. Synthesis of Hydroxytyrosyl Alkyl Ethers from Olive Oil Waste Waters. *Molecules*. 2009, 1762-1772.

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