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Human hydroxytyrosol's absorption and excretion from a nutraceutical

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ABSTRACT

Among the various (poly)phenols that are being sold as such or as part of a more complex mixture, hydroxytyrosol (HT) is the only one that bears a European Food Safety Authority health claim. Therefore, several HT-based products are being developed and sold and it becomes necessary to evaluate its accessibility following ingestion. Twenty-one volunteers were recruited for a randomized, crossover, placebo-controlled, and double-blind intervention study. We performed a Latin square design: after one-week washout, i.e. olive-free diet, subjects were randomly assigned to the placebo (maltodextrin), 5, or 25 mg/day HT group. Twenty-four hour urine samples were collected after the intervention week, and baseline urines were collected the week before the study and during periods of washout. The results show that HT given as the foremost component of a nutraceutical preparation is bioavailable and is recovered in the urine chiefly as sulphate-3′.

1. Introduction

The nutraceutical and functional food market is rapidly expanding and several new products enter the market on a daily basis (Mahabir, 2014; Tome-Carneiro & Visioli, 2015). Of note, such products are rarely tested in controlled human trial settings and the efficacy of individual molecules or raw extracts is often questionable. In addition, the bioavailability of individual molecules or active principle(s) is seldom assessed, in part because of technical limitations and lack of proper equipment.

Among the various (poly)phenols that are being sold as such or as part of a more complex mixture, hydroxytyrosol (HT) is the only one that bears a European Food Safety Authority health claim (EFSA Panel on Dietetic Products, 2011). Therefore, several HT-based products are being developed and sold (Visioli & Bernardini, 2011) and it becomes necessary to evaluate accessibility of HT following ingestion. It is noteworthy that HT bioavailability has been reported after extra virgin olive oil

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administration (Caruso, Visioli, Patelli, Galli, & Galli, 2001; Miro-Casas et al., 2003), yet never after the intake of HT-containing supplements, with the exception of one study with pure HT (Gonzalez-Santiago, Fonolla, & Lopez-Huertas, 2010).

In this study, we report the urinary excretion of HT (as such and as its metabolites) after its administration to healthy volunteers.

2. Materials and methods

2.1. Standards and chemicals

Hydroxytyrosol (HT, 98% purity) standard was purchased from Extrasynthese (France). HT 3′-O- and 4′-O- glucuronides (HT-G-3′ and HT-G-4′, 86% and 97% purity, respectively) were synthesized as previously described (Giordano, Dangles, Rakotomanomana, Baracchini, & Visioli, 2015). HT 3′-O-sulphate (HT-S-3′, 98% purity) standard was bought from Toronto Research Chemicals Inc. (Toronto, ON, Canada). Hydroxyphenylpropanol (HOPhPr, 99% purity), used as the internal standard (ISTD), was purchased from Sigma-Aldrich (St. Louis, MO, USA).

LC-grade solvents methanol and ACN were purchased from Scharlau Chemie, S.A. (Sentmenat, Spain). Ammonium acetate and glacial acetic acid were purchased from Panreac Química, S.A.U. (Castellar del Vallés, Spain). Ultrapure water (Milli-Q) was obtained from Millipore (Bedford, MA, USA).

The capsules that we administered were elaborated from an olive mill waste water extract preparation called Hytolive®, supplied by the company Genosa ID, S.L. (Madrid, Spain).

2.2. Subjects and study design

The study protocol was approved by the local ethics committee and written informed consent was obtained from all subjects prior to starting the trial. This work was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) and is registered at ClinicalTrials.gov (identifier: NCT02273622).

Samples of this research were obtained from a previous intervention study, whose objective was to evaluate the effect of HT on the gene expression of Phase II enzymes (Crespo et al., 2015). Briefly, twenty-one volunteers were recruited for a randomized, crossover, placebo-controlled, and double-blind intervention study. The design of this study is shown in Fig. 1. We performed a Latin square design: after one-week washout, i.e. olive-free diet, subjects were randomly assigned to the placebo (maltodextrin) group, 5 mg/day HT group, or 25 mg/day HT (Hytolive®) group. Baseline characteristics of participants and inclusion and exclusion criteria are given in detail in Supplementary Information 1 (S.I.1 in Appendix S1). Volunteers were given dietary guidelines (Supplementary Information 2, S.I.2 in Appendix S1) that included abstention from olive products and limitation of high-polyphenol foods and alcohol (Crespo et al., 2015). Twenty-four hour urine samples were collected after the intervention week, and baseline urines were collected the week before the study and during periods of washout, and immediately stored at –80 °C.

2.3. Pretreatment and processing of the urine samples

A total of 63 24-hour (from 21 volunteers, collected in the three experimental phases, after administration of the supplement) and 42 basal urine samples (collected during the final days of the second and third washout periods) were analysed.

All urine samples were thawed, vortexed, and centrifuged at 9000 × *g* for 5 min at 4 °C. The supernatant (20 µL) from each urine sample was diluted with 0.1% acetic acid by a factor of 10 (1:10 vol:vol) for detection of HT and its glucuronidate metabolites and by a factor of 50 (1:50 vol:vol) for its sulphates (HT-S-3′and HT-S-4′). Calibration standards of 5-10-25-50-100-250-500-1000 ng/mL for HT and 20-40-100-200-400-1000-2000-4000 ng/mL for HT-G-3′, HT-G-4′ and HT-S-3′ in blank human urine were processed like the 10-fold diluted samples. An internal standard (HOPhPr) was used at the final concentration of 500 ng/mL in all cases. Samples and calibration curves were distributed in 96-well plates and 2 µL of each were injected in randomized order.

2.4. Sample analysis

LC–MS/MS analysis of diluted samples was performed on the Agilent (Santa Clara, CA, USA) 1290 Infinity Binary LC system coupled to an AB SCIEX QTRAP® 6500 spectrophotometer. Acquity UPLC BEH C18 1.7 µm, 2.1 × 5 mm analytical column (Waters) at 40 °C and 1 mM ammonium acetate at pH 5.0 and 100% ACN as aqueous (A) and organic (B) mobile phases, respectively, were used for separation (Khymenets et al., 2011; Kotronoulas et al., 2013). Next, gradient elution (B% (v/v), t (min)) at flow of 0.4 mL/min was applied: (1%, 0–3); (1–20%, 3–3.2); (20%, 3.2–4.5); (20–95%, 4.5–4.8); (95%, 4.8–5.3); (95–1%, 5.3–5.5); (1%, 5.5–6.5). Common MS parameters were as follows: ion spray voltage (IS) –4500.00, source temperature (TEM) 600 °C, curtain

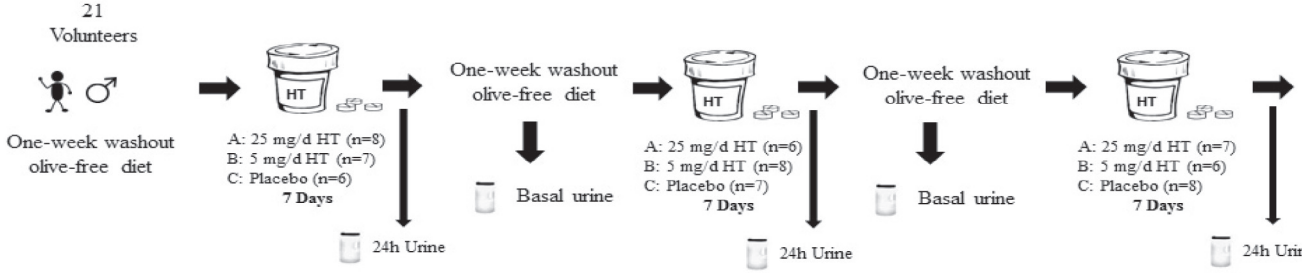

Fig. 1 – Study design.