HPLC Analysis of Trans-Resveratrol in Human Plasma After Red Wine Consumption

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Abstract: *Trans*-resveratrol (t-RES), a phenolic compound produced by several plants and present in wine, has been reported to be a potential chemopreventive agent for cardiovascular, cancer and neurodegenerative pathologies. Thus, understanding the plasma level *in vivo* of *trans*-resveratrol is the prerequisite to evaluate its potential health impact. Bioavailability studies mainly in animals or in humans using the pure compound at very high doses were performed. The objective of this present study was to detected *trans*-resveratrol in human plasma from two subjects who consumed 600 mL of red wine over 40 min. Plasma analyses were performed by HPLC and obtained results indicated the absence of t-RES in subject plasma at any time with limit of detection of 5 ng/mL. In conclusion, this study suggests that t-RES from red wine is poorly bioavailable and even an important red wine consumption does not make it possible to obtain detectable plasma concentrations of t-RES.

Keywords: *Trans*-Resveratrol, Bioavailability, Red Wine, Human Plasma, HPLC.

INTRODUCTION

Trans-resveratrol (3,5,4'-trihydroxystilbene; t-RES) is a polyphenol found in many plant, such as grapes, peanuts, berries, *polygonum* roots and traditional oriental medicine plants [1] with more important concentrations found in the red wines. Resveratrol can be found in the *cis*- or *trans*-form, with higher biological activity for the *trans*-isomer t-RES in [2]. It has been reported to have both anti-carcinogenic and cardio-protective activities, which could be attributed to its antioxidant and anticoagulant properties [3]. t-RES has been reported to inhibit platelet aggregation, alter eicosanoid synthesis and modulate lipoprotein metabolism [4-6]. Its potential cancer chemoprevention properties have been related to its capacity to inhibit diverse cellular events associated with the three major stages of carcinogenesis: initiation, promotion and proliferation [7]. Essentially, all these biological activities were reported *in vitro* with t-RES levels of 2 to 12 μg/mL [7-9]. However, its activity *in vivo* in human depends on its bioavailability and metabolism. The bioavailability studies reported in literature suffer from a drawback, since the amount of free t-RES used as pure compound (25 mg in hydro-alcoholic solution) is too large to be naturally assumed by drinking red wine. Thus, the aim of this study was to determine the presence of t-RES in human plasma after a red wine consumption. In order to quantify t-RES in human plasma, we used a method with a liquid/liquid extraction and HPLC with UV detection.

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SUBJECTS AND METHODS

Subjects and Samples

All subjects were healthy volunteers, non-smokers, moderate drinkers. Before the experiments they were subjected to a full physical examination with routine analysis of blood and urine to exclude hepatic and renal diseases, diabetes mellitus, dyslipoproteinemia, and hemopoietic disorders. Moreover, they were asked to sign an informed consent and not to consume alcoholic beverages for one week before the experiment. The study has been accepted by the Consultative Committee of the People Protection in the Biomedical Research in Auvergne (CCPPRB, n°U589). The average content of the free *t*-RES in wine (pinot noir, Santenay, France, 2000) was determined by applying the same method used for the t-RES determination in plasma (see below) and was found to be 1.89 ± 0.2 mg/L. Thus, during this experiment they ingested about 1.13 mg free t-RES.

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In practice, two healthy volunteers (man and woman) consumed 2 x 100 mL glasses of red wine at t0 and 4 wine glasses over 40 min with one glass (100 mL) every ten minutes. In parallel, blood samples (5 mL) were taken before drinking wine (t0, basal value), at 30 min (t30), 40 min (t40), 50 min (t50), 60 min (t60), 70 min (t70), 120 min (t2h), 240 min (t4h) and 48h (t48h). Blood samples were taken early in the morning after an overnight fast. After centrifugation for 10 min at 3000 rpm, plasma was collected and stored at -80°C until HPLC analysis. All this part was performed at the Centre de Recherche en Nutrition Humaine (Clermont-Ferrand, France).

Chemicals and Chromatographic Equipment

Carbamazepine, t-RES, monobasic sodium phosphate, ethyl acetate, acetonitrile and methanol were purchased from Sigma-Aldrich (Saint-Quentin-Fallavier, France). Water was filtered and deionized using a Milli-Q-UV plus system (Millipore, France). All chemicals were HPLC grade. We used a Hewlett-Packard 1100 series HPLC system with an UV-Vis detector. HPLC separation was obtained at 25°C using a 100 x 4.6 mm Inertsil octyl/150 Å, 5 µm analytical column (Interchim, France). The mobile phase consisted of 70% monobasic sodium phosphate (25 nM, pH 4.2) and 30% acetonitrile. The flow-rate was 1 mL/min.

Resveratrol Extraction and Quantitation

t-RES levels were determined in plasma by high pressure liquid chromatography according to the method of Zhu *et al.* (1999) with some modifications in chromatographic conditions [10]. Prior to application to the analytical column, 500 µl at plasma was precipated with 250 µl of 0.5 N sodium hydroxide. Extraction was performed with 6 mL ethyl acetate, mixed 10 min and centrifuged for 10 min at 3000 rpm. The organic layer was dried with a nitrogen evaporator. The dried residue was reconstituted with 150 µl of mobile phase and 80 µl of each sample was injected into the HPLC. For the quantitative analysis of t-RES, calibration curves were constructed by plotting peak area ratio of t-RES to the 3µg/mL carbamazepine internal standard against the analyte concentration. The absolute recovery was determined by comparing the peak height of extracted quality control samples prepared in human plasma, with the peak height of solutions prepared at the same concentrations. The analysis was done in six replicates at concentration levels of 20 ng/ml. The recovery of trans-resveratrol from spiked plasma sample of 20 ng/ml was 89.8 ± 6.6%. Linear regression analysis of these standard curves showed a correlation coefficient of r^2 = 0.9880 (SD: 0.0082). Retention times for t-RES and carbamazepine were 3.9 and 6.1 min, respectively.

RESULTS AND DISCUSSION

Various times used in this study are justified by a plasmatic peak of t-RES observed at 30 min after oral absorption of t-RES-rich solution. Goldberg *et al.* reported that 30 min after ingestion of 25 mg t-RES in three different matrices, the compound in its free form reached a peak concentration in the plasma that decreased during the next hours [11]. Trace amounts of free t-RES (<5 ng/mL) were also detected by Walle *et al.* in plasma of subjects after a 25 mg resveratrol oral dose [12]. Several *in vitro* as well as *in vivo* studies have reported that RES in its *trans* configuration is the most efficient in disease prevention [2]. Thus, in this study, we report t-RES concentration in human plasma analyzed by HPLC as previously described [10]. This method provides a simple and sensitive procedure for the quantitation of t-RES in human plasma samples. This method gives good linearity and reproducibility with detection and quantitation limits of 5 and 20 ng/mL, respectively. In comparison, using gas chromatography with mass selective detection, the concentrations of t-RES in blood serum, plasma and urine, as well as whole blood, have been measured after their oral and intragastric administration, respectively, to humans and rats. The limit of detection did not exceed 0.01 µg/l and the limit of quantitation did not surpass 0.1 µg/l [13]. Another method to measure *trans*-resveratrol in the serum of rats utilized HPLC on a C18 column followed by photodiode array detection in the UV range [14]. The limit of detection was 20 µg/l. A similar method was used by Kuhnle *et al.*, who also performed analyses based on LC/MS/MS technology [15].

In this present study, resveratrol was never found in any analyzed plasma samples and at any time. Examples of chromatograms are shown in Fig. (**1**). The absence of t-RES in all serum samples collected after wine ingestion could be explained considering literature data which indicate that, when absorbed, resveratrol is rapidly cleared through the glucuronidation and sulfation pathways [11, 12, 16] and rapid elimination in the urine [17]. There is evidence that the major form of resveratrol transferred across the rat intestinal epithelium into the blood stream is resveratrol glucuronides [18]. Glucuronidation and sulfation of resveratrol by human liver microsomes have been also reported and resulted in the formation of 3-*O*-glucuronide and 4'-*O*-glucuronides [19]. A recent study showed rapid metabolism and accumulation of t-RES essentially in liver [20]. In the other hand, RES could interact with serum proteins. Indeed, after incubation of 5 µM (~1.1µg/mL) t-RES with cell culture medium containing 10% fetal calf serum, all the t-RES was bound to proteins after 24 h, with albumin being the major candidate [21]. Thus, after absorption, the majority of t-RES reaching the plasma seems to be bound or metabolized.

Red wine was previously described to be a poorer source of flavonols in human [22]. Thus, the absence of t-RES could be also explained by its poor bioavailability from red wine. Our results were in accordance with the first study of t-RES bioavailability in humans from a moderate consumption of red wine. This study reported a high inter-individual in the absorption, and a rapid clearance of the absorbed t-RES. In the serum samples of 14 subjects of 25 tested, RES was never found in any form at any time, whereas in 11 of 25 at lest one serum sample was positive for free t-RES or a glucuronidated derivative. In any case, the detected amounts of free t-RES were always very low, in many cases below the limit of quantification [14].

In conclusion, this study suggests that red wine is a poorer source of t-RES. In addition, an important red wine consumption does not make it possible to obtain sufficient plasmatic concentrations to be detected by the present method. Thus, the reported data raises some doubts about the well-known beneficial health effects due to the presence of t-RES.

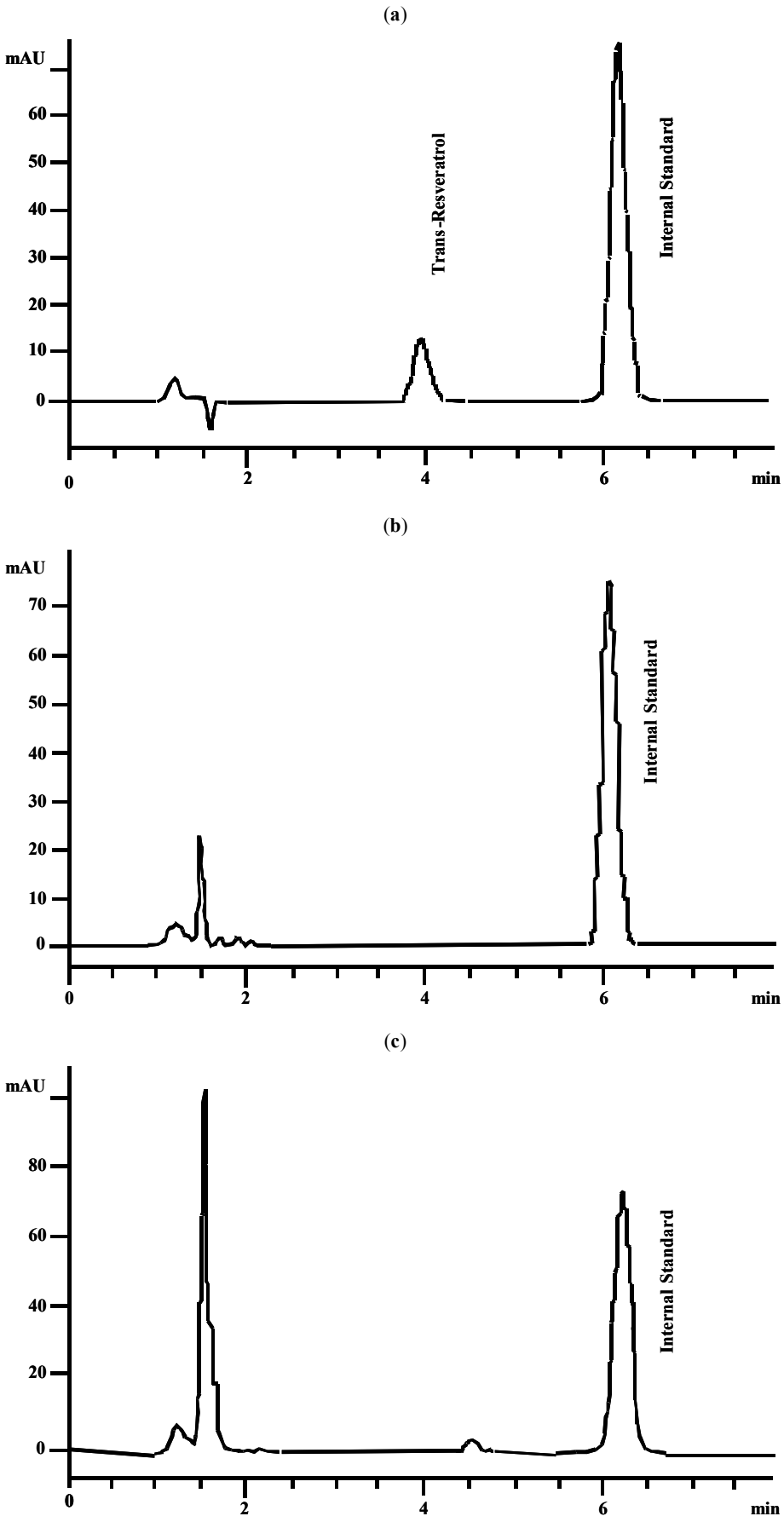

Fig. (1). Examples of chromatograms from extracted human plasma from sample with 200 ng/mL t-RES (**a**), from extracted human plasma at t30 (**b**) and t2h (**c**).

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