IN VITRO GLUCURONIDATION OF *trans*-PICEID AND *trans*-PICEATANNOL BY HUMAN LIVER MICROSOMES

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Abstract

Aims : The aim of the present investigation was to establish glucuronidation of *trans*-resveratrol derivates in the liver. Stilbenes are naturally occurring polyphenolic compounds which have been reported to have potential preventive activities in human diseases. *Trans*-stilbenes, mainly found in grapes and red wine, are important in terms of biological activities. However, little is known about the metabolism of these compounds in human.

Methods and results : The glucuronoconjugation of stilbenes was investigated using human liver microsomes and the structure of new metabolites was characterized by LC-MS and proton NMR. For the first time, the structure of the metabolites of *trans*-piceid and *trans*-piceatannol was established. The reaction led to the formation of two glucuronides for *trans*-piceid and three for *trans*-piceatannol.

Significance and impact of study: This study is of particular relevance since the phenolic substances of red wine (especially stilbenes) might be responsible for the potential beneficial effects of moderate and regular wine consumption.

Key words: *trans*-stilbenes, glucuronidation, human liver microsomes, LC-MS, proton NMR

Résumé

Objectif : L'objectif de cette étude est de déterminer la glucuronidation de dérivés du *trans*-resvératrol dans le foie. Les stilbènes sont des composés polyphénoliques naturels pouvant potentiellement prévenir certaines maladies humaines. Les *trans*-stilbènes, principalement trouvés dans le raisin et le vin rouge, sont importants du point de vue de leurs activités biologiques. Cependant il y a peu de connaissances sur le métabolisme de ces composés chez l'Homme.

Méthodes et résultats : La glucuronoconjugaison des stilbènes a été étudiée en utilisant des microsomes de foie humain et la structure des nouveaux métabolites a été caractérisée par LC-MS et RMN du proton. Pour la première fois, la structure des métabolites du *trans*-picéide et du *trans*-picéatannol a été établie. La réaction conduit à la formation de deux glucuronides pour le *trans*-picéide et trois pour le *trans*-picéatannol.

Signification et impact de l'étude : Cette étude est particulièrement intéressante dans la mesure où les composés phénoliques du vin rouge (notamment les stilbènes) pourraient être responsables des effets bénéfiques potentiels d'une consommation modérée et régulière de vin.

Mots-clés : *trans*-stilbènes, glucuronidation, microsomes de foie humain, LC-MS, RMN du proton

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INTRODUCTION

Trans-stilbenes are dietary phenolic compounds and the main sources of these substances are grapes and red wine. *Trans*-resveratrol and *trans*-piceatannol have been reported to have anti-carcinogenic, anti-oxidative and cardioprotective properties. However, these two aglycones are present in low concentrations in red wine compared to those of their glucosides (*trans*-piceid and *trans*-astringin), which may be more than 10-fold greater and also present activities (Vitrac *et al.*, 2007). Among these stilbenes, *trans*-resveratrol is widely investigated and numerous studies have shown that it is subjected to metabolic conversion in human. Resveratrol can be conjugated (glucuronide, sulfate) in liver or intestine and can also be metabolized to *trans*-piceatannol (Aumont *et al.*, 2001). By contrast, circulating forms (metabolites, native forms) of these other stilbenes have received less attention. Recently we studied the transport and metabolism of *trans*-piceid in human intestinal Caco-2 cells and showed that *trans*-piceid is transported across the enterocytes or is deglycosylated inside the cells into *trans*-resveratrol (Henry-Vitrac *et al.*, 2006).

The aim of the present investigation was to establish glucuronidation of three *trans*-resveratrol derivates, *trans*-piceid, *trans*-piceatannol and *trans*-astringin, using human liver microsomes.

MATERIALS AND METHODS

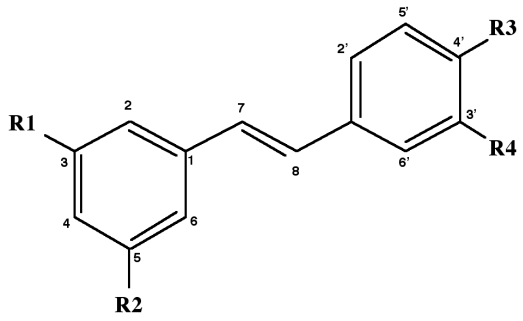
Trans-astringin and *trans*-piceid were isolated from *Vitis vinifera* cell suspension cultures by a combination of chromatographic techniques and were characterized by spectrometric methods. Piceatannol and resveratrol were supplied by Sigma Chemical Co. (Saint-Quentin-Fallavier-France).

Pooled liver microsomal preparations were prepared by standard differential methods with human liver fragments from several donors (study in agreement with the ethic committee of University Bordeaux II). Test compounds were incubated with pooled liver microsomal preparations in the presence of UDP-glucuronic acid, saccharonolactone and Tris-HCl/$MgCl_2$ at 37 °C, and the reaction was terminated after 60 min by addition of HCl. Samples were analyzed for stilbene metabolite(s) using reversed-phase high-performance liquid chromatography (HPLC). Then glucuronides were separated by semi-preparative HPLC and their structure was characterized by proton NMR. Molecular weight was confirmed by negative ion electrospray mass spectrometric analysis.

RESULTS AND DISCUSSION

We found for the first time that *trans*-piceatannol and *trans*-piceid are metabolized *in vitro* by human liver

Table 1 - Deducted structure of *trans*-piceid and *trans*-piceatannol glucuronides from NMR analysis.



	R1	R2	R3	R4
***trans*-resveratrol**	-OH	-OH	-OH	-H
***trans*-piceid**	-Glc	-OH	-OH	-H
-5-*O*-glucuronide	-Glc	-Glc-CO_2	-OH	-H
-4'-*O*-glucuronide	-Glc	OH	-Glc-CO_2	-H
***trans*-piceatannol**	-OH	-OH	-OH	-OH
-3-*O*-glucuronide	-Glc-CO_2	-OH	-OH	-OH
-3'-*O*-glucuronide	-OH	-OH	-OH	-Glc-CO_2
-4'-*O*-glucuronide	-OH	-OH	-Glc-CO_2	-OH

microsomes. In the presence of each *trans*-stilbene, the reaction led to the formation of several peaks: two for *trans*-piceid and three for *trans*-piceatannol. These peaks were absent when UDP-glucuronic acid was omitted and disappeared after hydrolysis by ß-glucuronidase, demonstrating that each was a ß-D glucuronide. The results indicate that the *in vitro* glucuronidation of the two *trans*-stilbenes by human liver microsomes led to the formation of mono-*O*-glucuronides, whose structure was characterized by LC-MS and proton NMR. All compounds and their metabolites are presented in table I. The apparent affinity of UDP-glucuronosyltransferases (UGTs) followed the order 3>4' for *trans*-resveratrol (Aumont *et al.*, 2001), 5>4' for *trans*-piceid, and 3>4'>3' for *trans*-piceatannol. Moreover, *trans*-piceid was hydrolyzed in the human liver microsomes, leading to *trans*-resveratrol, which is also a source of molecules having biological activities.

The glucuronidated conjugates of *trans*-astringin were not identified, indeed the quantity of these supposed glucuronides could be too low. Nevertheless, in the presence of *trans*-astringin, we detected its aglycone, *trans*-piceatannol, as for *trans*-piceid, we observed deglycosylation.

In human, several studies have shown that glucuronidation catalyzed by specific UGTs represents a major conjugative pathway for most exogenous polyphenols. The liver represents the main site of these biotransformations. It was shown that morphine-glucuronide is more potent than the native form itself and contributes to the analgesic effect of morphine (Lötsch and Geisslinger, 2001). Few studies on the biological activities of metabolites of polyphenols have been performed due to the difficulties inherent in producing sufficient samples to analyze. Some results showed that the glucuronides of quercetin can present biological activities but in the majority of cases, these effects are lesser than with the native form. Glucuronoconjugation usually leads to the formation of inactive compounds, which subsequently undergo renal and biliary elimination by increasing their hydrophilicity. However, these glucuronides are occasionally secreted *via* the biliary route into the duodenum, where they are re-activated by enzymatic hydrolysis that removes the glucuronate moiety, after which they may be reabsorbed. This entero-hepatic recycling may lead to the prolonged presence of a potential source of active polyphenols in human and therefore produce biological activities. Moreover, the glucurono-conjugates circulating in the blood might be hydrolyzed through the direct action of glucuronidases present in target tissues (in much greater proportion in cancerous tissues than in healthy samples) (Wang *et al.*, 2004).

In conclusion, this study shows that metabolism of stilbenes in the liver leads to the formation of glucuronides for *trans*-piceid and *trans*-piceatannol and that hydrolysis of the glucosides *trans*-piceid and *trans*-astringin, increases the circulating active forms *trans*-resveratrol and *trans*-piceatannol, respectively.

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BIBLIOGRAPHICAL REFERENCES

AUMONT V., KRISA S., BATTAGLIA E., NETTER P., RICHARD T., MÉRILLON J.M., MAGDALOU J. and SABOLOVIC N., 2001. Regioselective and stereospecific glucuronidation of *trans*- and *cis*-resveratrol in human. *Arch. Biochem. Biophys.*, **393**, 281-289.

HENRY-VITRAC C., DESMOULIÈRE A., GIRARD D., MÉRILLON J.M. and KRISA S., 2006. *Trans*port, deglycosylation, and metabolism of *trans*-piceid by small intestinal epithelial cells. *Eur. J. Nutr.*, **45**, 376-382.

LÖTSCH J. and GEISSLINGER G., 2001. Morphine-6-glucuronide: an analgesic of the future? *Clin. Pharmacokinet.*, **40**, 485-499.

VITRAC X., MONTI J.P., VERCAUTEREN J., DEFFIEUX G. and MÉRILLON J.M., 2002. Direct liquid chromatographic analysis of resveratrol derivatives and flavanonols in wines with absorbance and fluorescence detection. *Anal. Chim. Acta*, **458**, 103-110.

WANG L.X., HEREDIA A., SONG H., ZHANG Z., YU B., DAVIS C. and REDFIELD R., 2004. Resveratrol glucuronides as the metabolites of resveratrol in humans: Characterization, synthesis, and anti-HIV activity. *J. Pharm. Sci.*, **93**, 2448-2457.