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Research Paper

In-vitro sulfation of piceatannol by human liver cytosol and recombinant sulfotransferases

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Abstract

Objectives The aim of this study was to investigate the concentration-dependent sulfation of piceatannol, a dietary polyphenol present in grapes and wine and known for its promising anticancer and anti-inflammatory activity.

Methods Sulfation of piceatannol was investigated in human liver cytosol as well as using a panel of recombinant sulfotransferase isoforms. Furthermore, the chemical structures of novel sulfates were identified by liquid chromatography/mass spectrometry (LC/MS).

Key findings In the presence of 3′-phosphoadenosine-5′-phosphosulfate, three metabolites could be detected whose structures were identified by LC/MS/MS as piceatannol disulfate (M1) and two monosulfates (M2, M3). The kinetics of M1 formation exhibited a pattern of substrate inhibition with a K_i of 21.8 ± 11.3 μM and a V_{max}/K_m of 7.63 ± 1.80 μl/mg protein per min. Formation of M2 and M3 showed sigmoidal kinetics with apparent K_m and V_{max} values of 27.1 ± 2.90 μM and 118.4 ± 4.38 pmol/mg protein per min, respectively, for M2; and 35.7 ± 2.70 μM and 81.8 ± 2.77 pmol/mg protein per min, respectively, for M3. Incubation in the presence of human recombinant sulfotransferases (SULTs) demonstrated that M1 was formed equally by SULT1A1*1 and SULT1B1 and to a lesser extent by SULT1A1*2. M2 was preferentially catalysed by SULT1A1*2, 1A3 and 1E1. The formation of M3, however, was mainly catalysed by SULT1A2*1 and SULT1A3.

Conclusions Our results elucidate the importance of piceatannol sulfation in human liver, which must be taken into account in humans after dietary intake of piceatannol.

Keywords liver; piceatannol; sulfation

Introduction

Piceatannol (3,4,3′,5′-tetrahydroxy-*trans*-stilbene) is a naturally occurring polyphenol present in the skin of grapes, red wine, rhubarb, berries and sugarcane. It demonstrates a variety of biological and pharmacological activities including anti-inflammatory, antioxidative and immunosuppressive properties.[1–3] Piceatannol has pronounced inhibitory effects on cyclo-oxygenase 1 and 2[4] in addition to inducing apoptosis in various cancer cell lines including lymphoma,[5] colorectal cancer,[6] melanoma[7] and bladder cancer cells.[8] Furthermore, very recent experiments on human HL-60 promyelocytic leukaemia cells by Fritzer-Szekeres and colleagues[9] also showed pronounced inhibition of ribonucleotide reductase, inhibition of cell cycle progression, induction of apoptosis, and synergism with Ara-C.

Although the role of piceatannol in disease prevention has been extensively reported, published data concerning its metabolism is scarce. A recent study by Roupe *et al.*[10] on rats showed that piceatannol was exclusively metabolized to two glucuronide conjugates. These in-vivo data support in-vitro metabolism findings in rat liver microsomes, whereby glucuronidation is rapid and predominant.

No sulfated conjugation products of piceatannol have been identified thus far, although recently published data about the biotransformation of the analogous compound resveratrol (3,5,4′-trihydroxy-*trans*-stilbene) demonstrated approximately three-fold higher concentrations of resveratrol monosulfate as compared with resveratrol monoglucuronide in mouse blood after oral administration.[11] Resveratrol sulfation also seems to be the main metabolic pathway in humans. Walle and co-workers[12] were able to identify resveratrol monosulfate,

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presumably resveratrol-3-*O*-sulfate, as the main metabolite in plasma, as well as the existence of resveratrol monosulfate and two unidentified monoglucuronides in urine. No evidence of enzymatic oxidation by cytochrome P450 isoenzymes of resveratrol was found in plasma samples. The extremely rapid formation of resveratrol monosulfate either after intravenous or oral doses in rodents and humans indicates that sulfation is the main rate-limiting step in the bioavailability of resveratrol. The importance of sulfation over glucuronidation was also shown in one of our recent studies[13] investigating the metabolism of resveratrol in human breast cancer cell lines, leading to extensive intracellular conjugation of resveratrol-3-*O*-sulfate. Surprisingly, the formation of resveratrol glucuronides was below the detection limit.

Sulfation, catalysed by members of the cytosolic sulfotransferase enzyme family (SULTs), is an important metabolic pathway for steroid hormones, neurotransmitters, numerous xenobiotics and many drugs, as well as for dietary constituents.[14] These enzymes catalyse the transfer of the universal sulfuryl donor molecule 3′-phosphoadenosine-5′-phosphosulfate (PAPS) to the acceptor substrate.[15] In a recent in-vitro study we demonstrated, with a functional assay, that mono- and disulfation of resveratrol is catalysed by recombinant SULT1A1 and to a lesser extent also by SULT1A2, SULT1A3 and SULT1E1.[16]

Therefore, the aim of this study was to investigate the concentration-dependent sulfation of piceatannol using human liver cytosol as well as a panel of recombinant sulfotransferase isoforms. Furthermore, the chemical structures of novel sulfates should be identified by liquid chromatography/mass spectrometry (LC/MS).

Materials and Methods

Materials

Piceatannol (*trans*-3,4,3′,5′-tetrahydroxystilbene), sulfatase type V, from limpets (*Patella vulgata*), 3′-phosphoadenosine-5′-phosphosulfate (PAPS, 78% purity), dithiothreitol (DTT) and SULT1A1*2, 1A2*1, 1A3, 1E1 and 2A1 were obtained from Sigma-Aldrich (Munich, Germany). Methanol and water were of HPLC grade (Merck, Darmstadt, Germany). SULT1A1*1 and SULT1B1 were purchased from Cypex (Dundee, UK). Human liver cytosol (three males; mean age 37 years, range 16–66 years; 7-hydroxycoumarin sulfation, range 85–2200 pmol/mg per min) was obtained from BD Biosciences (Woburn, MA, US). All other chemicals and solvents were commercially available, of analytical grade, and used without further purification.

Metabolism of piceatannol by human liver cytosol

The reaction mixture contained 1–200 μM of piceatannol, 50 μM PAPS, 7.4 mg/ml DTT, 0.05 M potassium phosphate (pH 6.5) and human liver cytosol (5 mg/ml protein) in 150 mM KCl, 50 mM Tris, pH 7.5, and 2 mM EDTA in a total volume of 100 μl. PAPS and DTT stock solutions were prepared fresh each day. Reactions were initiated by adding PAPS, and the samples were incubated for 20 min at 37°C. After the reactions had been terminated by the addition of 200 μl methanol, the samples were centrifuged (13 000*g* for 5 min) and 80 μl of the supernatant was injected onto the HPLC column. Control experiments in the absence of PAPS were run in parallel. Piceatannol and its sulfates were determined using a Merck La Chrom System (Merck, Darmstadt, Germany) equipped with an L-7250 injector, an L-7100 pump, an L-7300 column oven (set to 15°C), a D-7000 interface and an L-7400 UV-detector, set to a wavelength of 310 nm. Chromatographic separation of piceatannol and its metabolites was performed on a Hypersil BDS-C_{18} column (5 μm, 250 × 4.6 mm i.d.; Thermo Fisher Scientific Inc., Waltham, MA, US), preceded by a Hypersil BDS-C_{18} precolumn (5 μm, 10 × 4.6 mm i.d.), at a flow rate of 1 ml/min. The mobile phase consisted of a continuous gradient as described previously.[13] Calibration of the chromatogram was accomplished using the external standard method. As standards of the metabolites were not available, quantification of metabolite concentrations was based on the assumption that the unknown metabolites had a similar molar extinction coefficient as did piceatannol. Linear calibration curves were derived from the peak area of piceatannol and its metabolites to the external standard piceatannol, using standard solutions of piceatannol, to yield a concentration range of 0.05–100 μg/ml. Linear regression analysis of the standard curve showed a correlation coefficient $r^2 = 0.999$. The precision of the assay (mean error, %) at the lowest concentrations of substrate was assessed by replicate analysis of cytosolic samples with known amounts of piceatannol. The inter-day values for the sulfate conjugates M1–M3 at 1 μM ranged from 7.2% to 12.5%, the intra-assay variability ranged from 3.4% to 9.5%.

SULT enzyme assays

To assay piceatannol metabolites, cytosolic extracts were prepared from Sf9 cells (containing cDNA for human SULT 1A1*2, 1A2*1, 1A3, 1E1 and 2A1) and *Escherichia coli* (containing cDNA for human SULT 1A1*1 and 1B1). Assays were performed in triplicate in a final volume of 100 μl containing 0.1 M KPO_4 buffer (pH 7.4), 1 μl of piceatannol (final concentration, 10 or 60 μM), 10 μl of PAPS (50 μM) and 2.5–6.6 μl of enzyme protein (0.25 mg/ml). Reactions were incubated at 37°C for 20 min and terminated by the addition of 200 μl methanol. The reaction mixtures were centrifuged at 13 000*g* for 5 min, and 100 μl of the clear supernatant applied onto an HPLC column. Sulfation of piceatannol was not observed when using control cytosol from Sf9 insect cells or *E. coli* containing the vector only. Enzyme kinetics for piceatannol sulfation by SULT1A1*1, 1A2*1, 1B1 and 1E1 were evaluated by incubation of the recombinant enzymes with piceatannol (1–600 μM) under conditions identical to those mentioned above.

Structural identification of piceatannol metabolites

After a 20-min incubation of human cytosol or recombinant SULTs, 2 μl of sulfatase (20 U) was added to 100 μl of the corresponding reaction mixtures and further incubated at 37°C for 24 h. The reactions were stopped by addition of methanol and the samples analysed as mentioned above. To distinguish between mono- and disulfation of piceatannol, liquid chromatography-mass spectrometry (LC/MS) measurements were performed with a 1100 Series HPLC system (Agilent,

Waldbronn, Germany) coupled to a G1315B DAD detector and QTrap 4000 LC/MS/MS system (Applied Biosystems, Foster City, CA, US) equipped with a TurboIonSpray ESI source. ESI-MS/MS was performed in negative polarity with the following settings: source temperature 550°C, curtain gas 10 psi (99.5% nitrogen), ion source gas 1 (sheath gas) 30 psi, ion source gas 2 (drying gas) 50 psi, ion spray voltage –4000 V, collision gas (nitrogen) high. The optimization of the analyte-dependent MS/MS parameters was performed by direct infusion of piceatannol (dissolved in 1 ml MeOH) into the mass spectrometer using a 11 Plus syringe pump (Harvard Apparatus, Holliston, MA, US). The injection volume was 20 μl. Column, mobile phase, gradient and flow rate were identical to those used for the analytical HPLC assay (see above).

Data analysis

Each incubation step in the various sulfation assays was performed at least in triplicate, and the results were expressed as means ± SD. The data were fitted to Michaelis–Menten (hyperbolic), substrate inhibition and Hill (sigmoidal) models, and further analysed using Eadie–Hofstee plots. The coefficient of determination (R^2) and visual inspection of the Eadie–Hofstee plots were used to determine the quality of a fit to the specific model. Kinetic parameters were estimated using the Prism program (version 5.0; GraphPad Software Inc., San Diego, CA, US) for Michaelis–Menten (Equation 1) and substrate inhibition kinetics (Equation 2) and the module Enzyme kinetics of the program SigmaPlot 2000 (version 6.01; SPSS Inc., Chicago, IL, US) for sigmoidal curves (Equation 3):

$$V = V_{max} \bullet S/(K_m + S) \quad (1)$$

$$V = V_{max}/(1 + K_m/S + S/K_i) \quad (2)$$

$$V = V_{max} \bullet S^n/(S_{50}^n + S^n) \quad (3)$$

where V is the rate of reaction, V_{max} the maximum velocity, K_m the Michaelis constant, S the substrate concentration, n the degree of sigmoidicity and K_i the inhibition constant. The enzymatic efficacy, which is defined as the V_{max}/K_m ratio, quantifies the sulfation capacity and corresponds to the intrinsic clearance.

Results

Piceatannol metabolism by human liver cytosol

Liver cytosol from three individual human livers was incubated with piceatannol (10 μM) for 20 min and subsequently analysed by HPLC. A typical HPLC chromatogram from these experiments is shown in Figure 1. In the presence of PAPS, three metabolites were detected in addition to piceatannol (t_r = 30.5 min): M1 (t_r = 20.0 min), M2 (t_r = 25.4 min) and M3 (t_r = 26.9 min).

Kinetics of piceatannol sulfation in human liver cytosol

Formation of M1–M3 was linear with time, up to 20 min, and with respect to cytosol protein concentrations of 2–10 mg/ml (data not shown). Figure 2 shows the sulfation of piceatannol with the respective Eadie–Hofstee plots. The kinetic constants for these reactions were estimated using piceatannol concentrations of 1–200 μM (see Table 1).

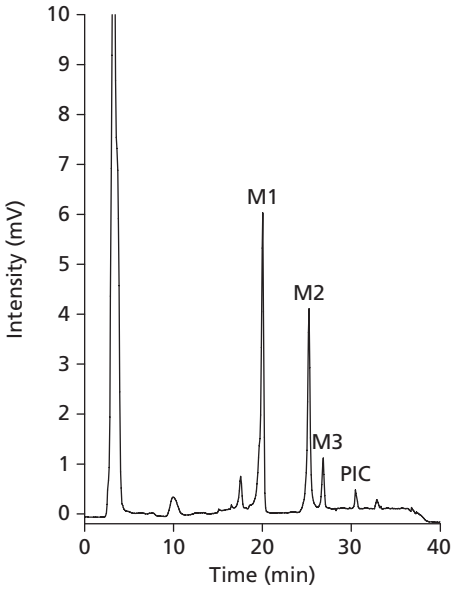

Figure 1 Representative HPLC chromatogram of piceatannol (PIC; 10 μM) and its three metabolites M1–M3 in human liver cytosol. For details see Materials and Methods.

As shown in Figure 2a, the apparent enzyme kinetic parameters for M1 were estimated by fitting to the substrate inhibition equation (Equation 2) (R^2 = 0.58). The substrate inhibition profile was further confirmed by an Eadie–Hofstee plot, which showed a characteristic hook.[17] In contrast to M1, the kinetic profile of M2 formation was best characterized by the atypical Hill model (R^2 = 0.88), which could be further confirmed by the Eadie–Hofstee plot (Figure 2b). Sigmoidicity of the curve was pronounced as described by a mean Hill coefficient (n) of 3.4. Enzyme kinetics for the formation of M3 also showed a sigmoidal kinetic profile (R^2 = 0.92), with a degree of sigmoidicity (n) of 3.0, as demonstrated by the atypical profile of a curved Eadie–Hofstee plot (Figure 2c).

Sulfation of piceatannol by recombinant SULTs

The structure of piceatannol suggests that one or more of the human SULTs involved in phenol or estrogen conjugation may readily sulfate this compound. Therefore, the ability of seven recombinant human SULTs to conjugate piceatannol at concentrations of 10 and 60 μM was investigated. This initial screening showed that SULT1A1*1, 1A1*2, 1A2*1, 1A3, 1B1 and 1E1 sulfated varying extents to up to three metabolites (Figure 3a, b). At 10 μM piceatannol, 37.8% of M1 is catalysed by SULT1A1*1, 40.0% by SULT1B1 and only 13.4% by SULT1A1*2. SULT1A2*1, 1A3 and 1E1 each only contribute to approximately 3% of the total M1 sulfation. M2 is formed by SULT1E1 (32.3%), 1A3 (26.8%), 1A1*2 (24.4%), and to a minor extent (4–6%) also by SULT1A1*1, 1A2*1 and 1B1. The formation of M3 is mainly catalysed by SULT1A2*1 (43.7%) and SULT1A3 (24.4%), whereas SULT1A1*1,

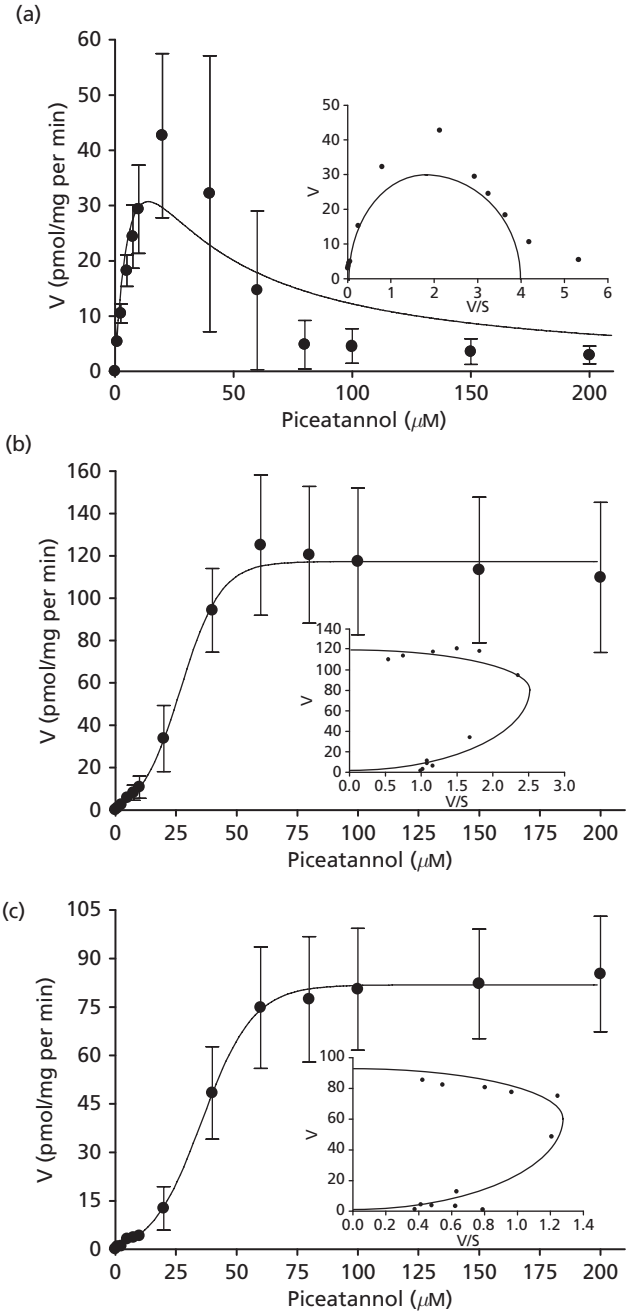

Figure 2 Kinetics of M1 (a), M2 (b) and M3 (c) formation in human liver cytosol normalized to protein content as a function of piceatannol concentration. Eadie–Hofstee plots are shown as insets on each graph. Human liver cytosol was incubated with piceatannol for 20 min at 37°C in the presence of PAPS. Data are expressed as means ± SD, n = 3, of individual preparations.

1A1*2, 1B1 and 1E1 exhibited low catalytic activity for the formation of M3.

Using 60 μM piceatannol, however, the formation rate for SULT1A1*1 was more than 26-fold higher for M2 than for M1 (6558 and 249.4 pmol/mg per min), indicating substrate inhibition (Figure 3b). For SULT1A1*2, the preference of M2 sulfation was even more significant: the formation of M1 at 60 μM piceatannol was now below the detection limit. A less pronounced shift from M1 to M3 was seen for SULT1B1 (1873 and 9559 pmol/mg protein per min). No formation of M1–M3 was seen with SULT2A1 at either low or high piceatannol concentrations.

Kinetics of piceatannol sulfation with recombinant SULT1A1*1, 1A2*1, 1B1 and 1E1

A comparative kinetic analysis was performed using piceatannol concentrations ranging from 1 to 600 μM to investigate, in greater detail, the catalytic activity of SULT1A1*1, 1A2*1, 1B1 and 1E1 for piceatannol sulfation. Kinetic data are reported in Tables 2 and 3.

The formation of M1 by the four recombinant SULTs fitted to the substrate inhibition model (Equation 2). The maximal velocity of formation (V_{max}) of M1 was significantly lower for SULT1A2*1 and SULT1E1 than that for SULT1A1*1 and SULT1B1. K_i values also differed significantly among the four investigated SULT isoenzymes. Interestingly, the SULTs with the highest V_{max} values showed the lowest K_i estimates.

The formation of M2 by SULT1A1*1 and 1B1 followed atypical enzyme kinetics, which were best fitted to the Hill equation, resulting in a slightly lower K_m value but 1.5- and 2.2-fold higher V_{max} and V_{max}/K_m values for SULT1A1*1 than for SULT1B1. The formation of M2 by recombinant SULT1A2*1 displayed Michaelis–Menten kinetics at 1–600 μM piceatannol. Only the formation of M2 by SULT1E1 exhibited inhibition kinetics when piceatannol concentration exceeded 20 μM.

The formation of M3 by SULT1A1*1 and SULT1B1 could again be best characterized by the atypical Hill model. The sigmoidicity of the curves was pronounced, as described by a mean Hill coefficient (n) of 4.6 and 2.0. Comparison of the kinetic estimates revealed 1.3- and 4.5-fold higher K_m and V_{max} estimates for SULT1B1 compared with those for the SULT1A1*1 isoenzyme. However, the formation of M3 by SULT1A2*1 followed classical Michaelis–Menten kinetics. Contrary to SULT1A1*1, SULT1A2*1 and SULT1B1, the formation of M3 by SULT1E1 displayed a substrate inhibition profile with low K_m and V_{max} but very high K_i values.

Identification of piceatannol metabolites

After incubation of human liver cytosol samples with sulfatase before HPLC analysis, M1 was no longer detectable. The concomitant increase in M2, M3 and the parent piceatannol indicates that M1 is a disulfate originating from the monosulfates M2 and M3 (data not shown). Structural identification of piceatannol sulfates was confirmed by LC-MS/MS. Negative ion mass spectra of piceatannol and its three biotransformation products showed stable molecular ions at m/z = 243, 323, 323, and m/z = 403 amu, with subsequent loss of 80 amu (SO_3 moiety) for M2 and M3 and 160 amu (two SO_3 moieties) for M1, in agreement with the molecular weight of piceatannol, piceatannol monosulfates and piceatannol disulfate (data not shown). Based on mass spectra, however, it was not possible to determine the point of attachment for sulfonation (Figure 4).

Table 1 Kinetic parameters of M1–M3 formation in human liver cytosol

Metabolite	Model	K_m (μM)	V_{max} (pmol/mg protein per min)	V_{max}/K_m (μl/min per mg)	*n* (Hill coefficient)	K_i (μM)
M1	Substrate inhibition	9.28 ± 4.05	70.8 ± 20.3	7.63 ± 1.80	n.a.	21.8 ± 11.3
M2	Hill	27.1 ± 2.90	118.4 ± 4.38	4.37 ± 2.32	3.4	n.a.
M3	Hill	35.7 ± 2.70	81.8 ± 2.77	2.29 ± 0.17	3.0	n.a.

Data are shown as means ± SD of three determinations.
n.a., not applicable.

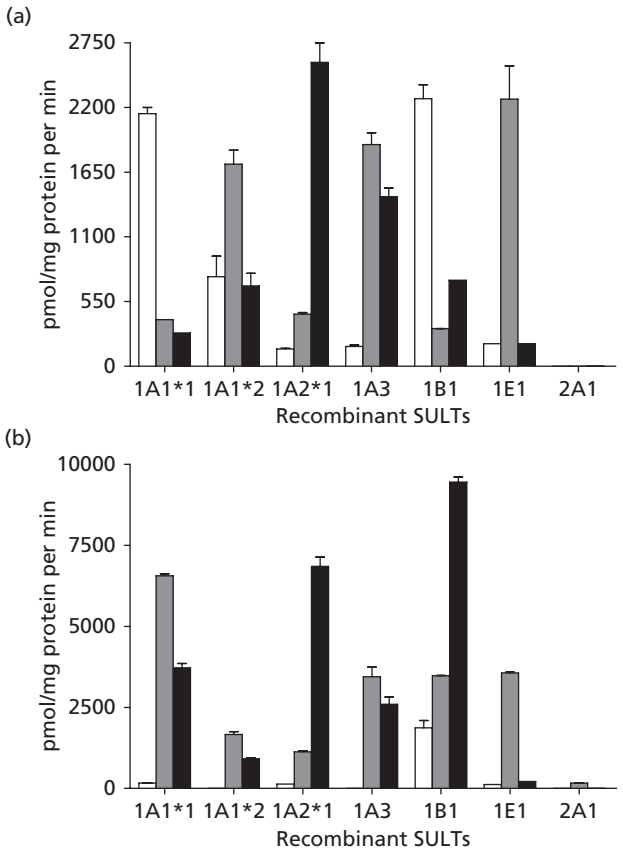

Figure 3 Rate of M1–M3 formation in recombinant human sulfotransferases (SULTs) normalized to protein content as a function of piceatannol concentration. Recombinant human SULTs were incubated with 10 μM (a) and 60 μM (b) piceatannol for 20 min at 37°C in the presence of PAPS. Open columns, M1; grey columns; M2, black columns, M3. Data are shown as means ± SD of three determinations.

Discussion

Sulfation is an important metabolic pathway in the intestine and human liver for a variety of drugs, endogenous substrates and dietary polyphenols. The process is mediated by members of the cytosolic sulfotransferase (SULT) family, increases the polarity of hydrophobic compounds and results, in many cases, in loss of biological activity. SULTs also play a role in the generation of bioactive compounds. Minoxidil,[18] triamterene,[19] cicletanine[20] and morphine,[21] in particular, are sulfated to more active compounds.

One of the purposes of this study was to investigate the ability of piceatannol to be sulfated by human liver cytosol and by SULT isoforms abundant in the gastrointestinal tract and liver, such as SULT1A1, 1A2, 1A3, 1B1, 1E1 and 2A1. Piceatannol is extensively metabolized in human liver to three conjugates identified by LC/MS/MS analysis and enzymatic hydrolysis with sulfatases, namely piceatannol disulfate (M1) and two monosulfates (M2 and M3). The formation of M1 clearly prevailed at lower substrate concentrations of 1–20 μM. However, at concentrations greater than 20 μM piceatannol, substrate inhibition was observed. This led to greater formation of the two monosulfates M2 and M3. Using a panel of recombinant SULTs, we were able to show that the formation of M1–M3 was highly dependent on substrate concentration. At 10 μM piceatannol, mainly SULT1A1*1 and SULT1B1 are responsible for the formation of M1 whereas SULT1A1*2, SULT1A3, and SULT1E1 are the major isoforms catalysing the formation of M2. M3 is predominantly conjugated by SULT1A2*1 and, to a lesser extent, by SULT1A3. At a higher piceatannol concentration (60 μM), the formation of M1 by SULT1A1*1, and to a lesser extent also by SULT1B1, was strongly inhibited, leading to substrate inhibition kinetics, with K_i values of 1.3 and 3.3 μM, respectively.

Less pronounced substrate inhibition was seen for M1 formation by SULT1A2*1 and SULT1E1 (K_i: 148.6 μM and 25.8 μM) possibly due to a significantly higher affinity to these isoenzymes (K_m: 1.02 μM and 4.47 μM), which may also explain the lower V_{max} values. The predominant role of SULT1A1*1 in the formation of M1 was confirmed when comparing its mean K_m values in liver cytosol and recombinant SULT1A1*1, which were nearly identical (liver cytosol: 15.8 μM; recombinant SULT1A1*1: 16.5 μM). Remarkably, SULT1E1 showed substrate inhibition profiles for all three piceatannol sulfates.

Our data demonstrated hepatic as well as extrahepatic sulfation of piceatannol. SULT1A1 levels are very high in the liver and also present at lower levels in many other tissues, whereas SULT1A3 expression is extremely high in the gut, detectable in several other extrahepatic tissues, and is essentially not expressed in the liver.[22] Lower mRNA levels have been found for SULT1A2 than for other SULT1A members in the liver, kidney, brain, ovary and some other sections of the gastrointestinal tract. As recent published data also show the expression of SULT1A2 protein in Caco-2 cells as well as in individual samples of the liver and caecum,[23,24] our study is indicative of the physiological role of SULT1A2 in piceatannol biotransformation. SULT1B1 mRNA has been primarily identified in the liver, small intestine, colon and in blood leucocytes.[25] Teubner and colleagues[23] reported a 17-fold higher expression of SULT1B1 in the ileum than in the liver. SULT1E1 is present in the intestine, liver protein and in hormone-dependent tissues

Table 2 Kinetic parameters of M1–M3 formation by recombinant human sulfotransferases SULT1A1*1 and SULT1A2*1

Metabolite model	SULT1A1*1			SULT1A2*1		
	M1 Substrate inhibition	M2 Hill	M3 Hill	M1 Substrate inhibition	M2 Substrate inhibition	M3 Substrate inhibition
K_m (μM)	291.2 ± 83.2	36.9 ± 2.10	34.3 ± 2.03	1.02 ± 0.21	51.3 ± 5.13	46.5 ± 5.26
V_{max} (pmol/mg protein per min)	89252 ± 26083	955 ± 230.2	4072 ± 89.4	181.3 ± 8.02	2155 ± 66.5	11732 ± 399.1
V_{max}/K_m (μl/min per mg)	306.5 ± 152.8	258.6 ± 150.7	118.6 ± 89.2	177.7 ± 32.5	42.0 ± 4.17	252.1 ± 127.3
n (Hill coefficient)	n.a.	7.3	4.6	n.a.	n.a.	n.a.
K_i (μM)	1.30 ± 2.47	n.a.	n.a.	148.6 ± 21.7	n.a.	n.a.

Data are shown as means ± SD of three determinations.
n.a., not applicable.

Table 3 Kinetic parameters of M1–M3 formation by recombinant human sulfotransferases SULT1B1 and SULT1E1

Metabolite model	SULT1B1			SULT1E1		
	M1 Substrate inhibition	M2 Hill	M3 Hill	M1 Substrate inhibition	M2 Substrate inhibition	M3 Substrate inhibition
K_m (μM)	254.0 ± 64.2	55.9 ± 7.50	46.7 ± 13.6	4.47 ± 1.11	142.2 ± 48.1	1.24 ± 0.28
V_{max} (pmol/mg protein per min)	75822 ± 21060	6447 ± 133.8	18525 ± 471.0	443.4 ± 54.5	39423 ± 17020	239.3 ± 10.3
V_{max}/K_m (μl/min per mg)	298.5 ± 116.9	115.4 ± 34.9	396.3 ± 181.3	99.1 ± 63.8	277.2 ± 123.7	193.8 ± 91.3
n (Hill coefficient)	n.a.	2.1	2.0	n.a.	n.a.	n.a.
K_i (μM)	3.33 ± 2.26	n.a.	n.a.	25.8 ± 5.31	10.6 ± 8.93	729.0 ± 207.3

Data are shown as means ± SD of three determinations.
n.a., not applicable.

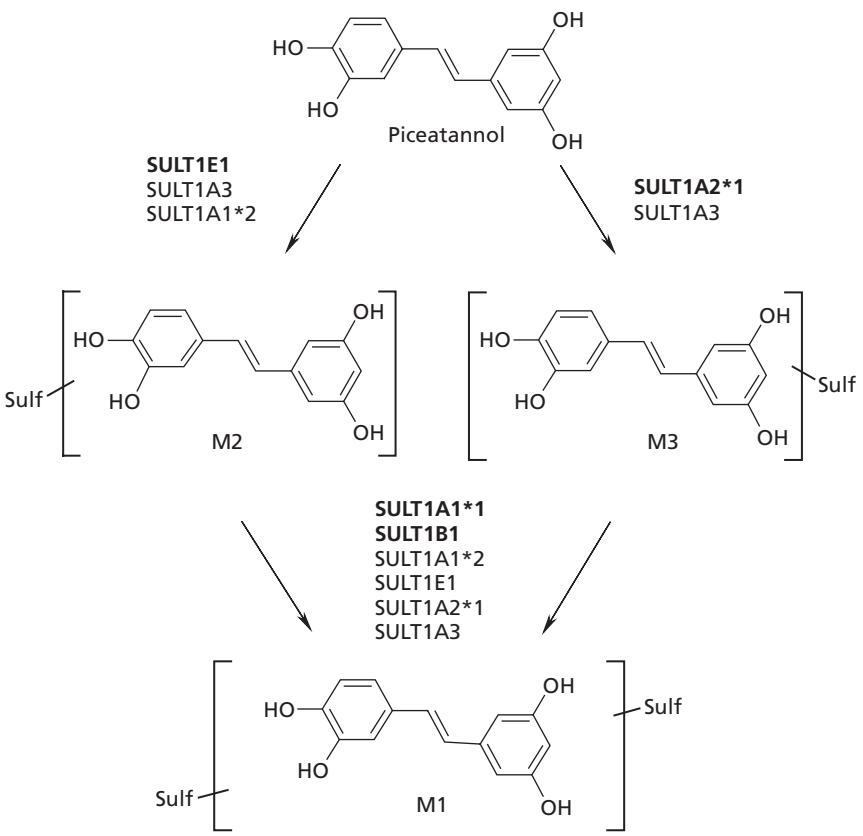

Figure 4 Proposed metabolic pathway of piceatannol (10 μM) by human liver cytosol and recombinant sulfotransferases (SULTs). SULT enzymes shown in bold are those that contribute most to sulfate formation.

(such as the endometrium). High levels of SULT2A1 proteins are present in adrenal glands and liver while low levels are found in the small intestine; SULT2A1 proteins have not been found in any other tissues. During absorption, piceatannol may be sulfated by several intestinal SULT enzymes, which may contribute to the metabolism of piceatannol.

However, Roupe and co-workers[10,26] found no piceatannol sulfates in rat plasma after intravenous administration of 10 mg/kg piceatannol. This discrepancy may be due to the use of phosphoric acid as a constituent of the mobile phase in the HPLC system, because recent data from our laboratory demonstrated pronounced hydrolysis (> 70%) of all piceatannol sulfates during 30 min at room temperature under acidic conditions (0.02–0.1% acetic acid or phosphoric acid, respectively). No hydrolysis was observed at pH 7.4 (data not shown). Furthermore, initial piceatannol concentrations in the rat plasma exceeded 70 μM, which is far beyond the observed inhibition concentration of M1 formation. Based on the high formation rates of M1–M3 in liver cytosol, we believe that all three conjugates should be detectable in human plasma and urine after oral administration.

Conclusions

Our data demonstrate that piceatannol is extensively sulfated in human liver, catalysed by SULT1A1*1, SULT1A2*1, SULT1B1 and SULT1E1. It is not yet known whether piceatannol conjugates also exhibit pharmacological activity. However, like the biologically inactive estrogen sulfate, which is cleaved by cellular sulfatases into the bioactive estradiol, piceatannol conjugates may also serve as inactive pools for piceatannol. Conjugation might also prevent piceatannol from enzymatic oxidation, extending its half-life in the cell and maintaining its biological properties.[27] Sulfation may therefore play a key role in the elimination of piceatannol in humans after oral uptake of dietary piceatannol.

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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