Hepatic Glucuronidation of Resveratrol: Interspecies Comparison of Enzyme Kinetic Profiles in Human, Mouse, Rat, and Dog

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Summary: The enzyme kinetic profiles of the formation of resveratrol-3-O-glucuronide (R3G) and resveratrol-4′-O-glucuronide (R4′G) by liver microsomes from humans, dogs, and rodents were investigated. Glucuronidation by human and dog liver microsomes to R3G and R4′G occurred for about 65% of applied resveratrol, and was significantly reduced to 10% when substrate concentration was increased 10-fold. In contrast, rodent microsomes glucuronidated about 90% of applied resveratrol independently of substrate concentration. Furthermore, in mouse and rat liver microsomes, resveratrol was almost exclusively conjugated at position 3, whereas human and dog livers also glucuronidated resveratrol at position 4′ (ratio R3G:R4′G = 5:1). Interspecies differences were also found when calculating the enzyme kinetic profiles of both conjugates. Formation of R4′G in human and dog microsomes followed Michaelis-Menten kinetics, while R3G showed substrate inhibition at higher resveratrol concentrations. In mouse and rat microsomes, however, both R3G and R4′G formation exhibited auto-activation kinetics. Formation of R3G and R4′G by recombinant UGT1A1 also showed substrate inhibition kinetics that led to decreased intrinsic clearance values, while UGT1A9-catalyzed glucuronidation demonstrated substrate inhibition kinetics at position 3 and Hill kinetics for the formation of R4′G. In conclusion, resveratrol glucuronidation exhibited species-dependent differences, with the dog as the animal model that most closely represents humans in terms of this process.

Keywords: hepatic metabolism; resveratrol; glucuronidation; species comparison; enzyme kinetics

Introduction

Resveratrol (trans-3,5,4′-trihydroxystilbene) is a natural compound produced in more than 70 plant species with high amounts found in grapes, berries, peanuts, and red wine. Over the last decade it has been shown that resveratrol has a wide variety of biological and pharmacological properties. In several in vitro and animal models, resveratrol was found to be active in the prevention and treatment of cancer, cardiovascular diseases, inflammation, ischemic injuries, and neurodegenerative diseases; it may also act as an antiobesity and antiaging compound. Resveratrol has demonstrated pronounced pharmacological activities in clinical studies; for example, it was effective for the reduction of tumor cell proliferation in colorectal cancer patients, the modulation of enzyme systems involved in carcinogen activation and detoxification, the increase of cerebral blood flow, and the suppression of oxidative and inflammatory stress. Several phase I/II clinical trials of oral resveratrol in cancer prevention and therapy are currently being performed.

The bioavailability of resveratrol, however, is low, due to extensive intestinal and hepatic metabolism. After oral administration of 25 mg resveratrol to human volunteers (red wine or as dietary supplement) only trace amounts of free resveratrol could be found in the plasma (<5 ng/ml). The discrepancy between the observed in vitro action and the
extensive biotransformation may be explained by the potential biological activity of the metabolites themselves. Indeed, there is evidence that resveratrol conjugates are biologically active. The 3-O-sulfated (R3S) and 4′-O-sulfated (R4S) conjugates exhibit biological activities known to be mediated by resveratrol, including induction of quinone reductase 1 (QR1), free radical scavenging, inhibition of cyclooxygenase (COX-1 and COX-2), alpha-induced NFκB activity, and activation of SIRT1.\textsuperscript{16,17} Resveratrol-3-O-glucuronide (R3G), on the other hand, showed higher antioxidant activity than the parent compound.\textsuperscript{18} These resveratrol metabolites may therefore also contribute to the various health benefits that were previously attributed only to resveratrol.

The biotransformation of resveratrol is a complex process. In humans, R3G, R3S, resveratrol 4′-O-glucuronide (R4G), and R4S are the most abundant conjugation products. In addition to these main mono-conjugates, various studies have also reported a variety of minor disulfates and diglucuronides.\textsuperscript{19,20} The variability becomes even greater when other species are examined; for example, in rats, the additional formation of trisulfates and sulfate-glucuronides is observed.\textsuperscript{21,22} Glucuronidation seems to be the major pathway in the phase II biotransformation of resveratrol. A study in humans also reports higher formation rates for resveratrol glucuronides compared to sulfates after ingestion of 250 ml of red wine (corresponding to 5.4 mg resveratrol).\textsuperscript{23} R3G was also the most abundant biotransformation product (maximal mean concentration: 34.74 µg/g) in the colorectal tissue of patients after eight daily doses of resveratrol at 0.5 or 1.0 g.\textsuperscript{6} In a repeat dose study using 0.5–5 g resveratrol/day for 29 days, the maximal plasma concentration of the resveratrol glucuronides was slightly below that of R3S for low doses, while glucuronidation prevailed when the dose was above 2.5 g.\textsuperscript{24} This is in accordance with our findings, as we observed in rat liver and human Caco-2 cells that the metabolism of resveratrol strongly depends on the applied dose. In both models, sulfates were the most abundant metabolites at lower concentrations, while R3G was the predominant conjugate at higher resveratrol concentrations due to substrate inhibition of sulfates.\textsuperscript{22,25}

The glucuronidation of many natural polyphenols is known to differ greatly between humans and other animals,\textsuperscript{26–28} but it is unknown whether this is also true for resveratrol. The objective of the present study was therefore to quantify and compare for the first time the formation of R3G and R4G in human, dog, mouse, and rat liver microsomes. Furthermore, the dose dependency of resveratrol glucuronidation was studied at the concentration levels used for in vivo studies.

Materials and Methods

Materials: Resveratrol (3,4′,5-trihydroxy-trans-stilbene), uridine 5′-diphosphoglucuronic acid (UDPGA), Triton X-100, Brij 35, Brij 58, digitonin, alamethicin, and β-glucuronidase type B-3 from bovine liver were obtained from Sigma-Aldrich (Munich, Germany). Methanol and water were of HPLC grade (Merck, Darmstadt, Germany). Recombinant human UDP-glucuronosyltransferase (UGT) 1A1, 1A3, 1A4, 1A6, 1A7, 1A9, 1A10, 2B7, and 2B12 (BD Gentest Supersomes) as well as human liver microsomes and pooled rat (male Wistar Han), mouse (male CD-1), and dog (male beagle) liver microsomes were purchased from BD-Gentest (Woburn, MA, USA). The protein contents and enzyme activities were used as described by the manufacturer. All other chemicals and solvents were commercially available, of analytical grade, and used without further purification.

Glucuronidation of resveratrol: Incubation conditions were initially optimized for linearity of glucuronide formation with respect to incubation time (60–300 min), microsomal protein concentrations (0.5–2 mg/ml), and activation of microsomes by Triton X-100, Brij 35, Brij 58, digitonin, and alamethicin (0.25, 0.5, and 1 mg/mg protein). For the subsequent assays, microsomes (0.5 mg protein/ml) were preincubated with Brij 58 (0.25 mg/mg protein) on ice. After 30 min, an incubation mixture containing magnesium chloride (10 mM); D-saccharic acid-1,4-lactone (5 mM); and resveratrol was added to 50 mM potassium phosphate buffer (pH 7.4) to yield a total volume of 250 µl. The reaction was started by the addition of 1 mM UDPGA, and samples were incubated for 60 min at 37°C. After the reactions were terminated by adding 500 µl chilled methanol, the samples were centrifuged (13000 g for 5 min) and 100 µl of the supernatant was injected onto the HPLC column. For each sample type, control experiments in the absence of UDPGA were run in parallel.

Quantification and identification of resveratrol and its glucuronides: The quantification of resveratrol and its glucuronides in the samples was performed by a sensitive reverse-phase HPLC analysis as previously described.\textsuperscript{29} Briefly, resveratrol and its glucuronides were separated on a Hypersil BDS-C18 column (5 µm, 250 × 4.6 mm I.D., Astmoor, England), preceded by a Hypersil BDS-C18 precolumn (5 µm, 10 × 4.6 mm I.D.), at a column temperature of 15°C using a mobile phase consisting of a continuous gradient mixed from 5 mM ammonium acetate/acetic acid buffer, pH 7.4, and methanol at a flow rate of 1 ml/min. Quantification of resveratrol and its glucuronides was monitored at 307 nm. Linear calibration curves were obtained to compare the peak areas of resveratrol and R3G to the external standards of resveratrol and R3G using standard solutions of these compounds to give a concentration range from 0.05 to 10 µg/ml (average correlation coefficients: >0.99). Because a standard for R4G was not available in adequate amounts, quantification of this conjugate was based on the assumption that the molar extinction coefficient of R4G is similar to that of R3G. The formation of resveratrol glucuronides was confirmed by co-incubating human liver microsomes with β-glucuronidase
type B-3 from bovine liver (200 units). Control samples were incubated in the absence of enzymes. Furthermore, the retention times of the two resveratrol conjugates were identical to those of R4′G and R3G standards isolated from the bile of Wistar rats.\textsuperscript{22}

**UGT reaction screening of resveratrol:** UGT screening of resveratrol was performed with microsomes prepared from lymphoblasts or Sf9 cells containing the cDNA for human UGT 1A1, 1A3, 1A4, 1A6, 1A7, 1A9, 1A10, 2B7, and 2B15. After preincubation with Brij 58 (0.25 mg/mg protein), microsomes (0.5 mg protein/ml) were incubated at 37°C with resveratrol (50 μM) and UDPGA (1 mM) under identical conditions to those mentioned above. Blank reactions contained buffer in place of substrate.

**Enzyme kinetics of resveratrol glucuronidation:** Apparent enzyme kinetic parameters for the formation of resveratrol glucuronides were determined with human, rat, mouse, and dog liver microsomes and human recombinant UGT1A1 (activity: 45 pmol/mg.min) and UGT1A9 (activity: 1960 pmol/mg.min) using various resveratrol concentrations (10–2000 μM in DMSO) at a protein concentration of 0.5 mg/ml. Incubation and analysis conditions were similar to those described above. Glucuronidation of resveratrol was omitted when control microsomes from cells containing only the vector were used.

**Data analysis:** Each incubation in the various glucuronidation assays was performed at least in triplicate (human liver microsomes in quintuplicate), and the results were expressed as mean ± SD. Kinetic parameters were estimated using the Prism program (version 5.0, GraphPad Software, San Diego, CA). The data were fitted to the following models:

1. The Michaelis-Menten model (hyperbolic)
   \[ V = V_{\text{max}} \times S / (K_m + S) \]  
   where \( V \) is the rate of reaction, \( S \) is the substrate concentration, \( V_{\text{max}} \) is the maximum enzyme velocity, and \( K_m \) is the Michaelis constant (substrate concentration needed to achieve a half-maximum enzyme velocity).

2. The substrate inhibition model
   \[ V = V_{\text{max}} \times S / (K_m + S \times (1 + S / K_i)) \]  
   where \( V_{\text{max}} \) is the maximum enzyme velocity if the substrate does not also inhibit enzyme activity and \( K_i \) is the substrate inhibition constant.

3. The Hill model (sigmoidal)
   \[ V = V_{\text{max}} \times S^n / (S_{50}^n + S^n) \]  
   where \( S_{50} \) is the substrate concentration resulting in 50% of \( V_{\text{max}} \) (analogous to \( K_m \)) and \( n \) is the Hill coefficient (= degree of sigmoidicity).

Kinetic profiles were further analyzed 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 a specific model. The enzymatic efficacy, which is defined as the ratio \( V_{\text{max}} / K_m \), quantifies the glucuronidation capacity and corresponds to the intrinsic clearance.

**Results**

**Formation of resveratrol glucuronides:** To assess hepatic glucuronidation of resveratrol, liver microsomes from five individual human livers and pooled rat, mouse, and dog liver microsomes were incubated with resveratrol for 60 min and subsequently analyzed by HPLC. In the presence of UDPGA, in addition to native resveratrol \( (t_c = 31.5 \text{ min}) \), both main glucuronide conjugates, resveratrol-4′-O-glucuronide (R4′G) \( (t_c = 18.6 \text{ min}) \) and resveratrol-3-O-glucuronide (R3G) \( (t_c = 22.6 \text{ min}) \), could be detected for all analyzed protein sources. Figure 1 shows a typical HPLC chromatogram from human liver microsomes. Structural identification of the biotransformation products was conducted by treatment with β-glucuronidase and comparison of the compound’s retention times with those obtained from standards of R3G and R4′G isolated from the bile of Wistar rats.\textsuperscript{22} Formation of R3G and R4′G increased linearly with time up to 300 min and with microsomal protein concentrations in the range 0.5–2 mg/ml. Among the detergents tested for UGT activation, Brij 58 (0.25 mg/mg protein) showed the highest formation rates. (Fig. 2).

**Species differences in resveratrol glucuronidation:** Liver microsomes were incubated with 100 or 1000 μM resveratrol for 60 min and formation of resveratrol glucuronides was quantified via HPLC analysis. In all species tested, formation of R3G and R4′G was observed, but at
different formation ratios. As shown in Figure 3, conjugation at position 3 was clearly favored by liver microsomes from all species tested. At 100 µM resveratrol, 50.1 ± 2.37% and 58.1 ± 1.19% of the applied dose was conjugated to R3G by human and dog microsomes, respectively, but only 11.3 ± 2.90% and 8.91 ± 1.11% was conjugated to R4'G. In contrast, glucuronidation at position 4 by rat and mouse microsomes was negligible (0.21 ± 0.01% and 1.99 ± 0.14% of applied dose, respectively). In these species, 92.9 ± 1.44% and 92.1 ± 2.00% of resveratrol was metabolized to R3G. However, when the resveratrol concentration was increased to 1000 µM, a pronounced reduction of R3G and R4'G formation to 9.11 ± 0.59% and 3.24 ± 0.27% in human liver and to 7.76 ± 0.27% and 1.95 ± 0.06%, respectively, in dog microsomes was observed. In mouse microsomes this reduction was less pronounced (R3G: 64.5 ± 6.69%; R4'G: 1.69 ± 0.08%). Interestingly, in rat liver, an increased resveratrol concentration did not alter glucuronidation at the 3 or 4' positions.

**Species differences in the kinetic profiles of resveratrol-3-O-glucuronidation:** The enzyme kinetic profiles for resveratrol glucuronidation at position 3 were evaluated using data obtained from incubations with 10–2000 µM resveratrol for 30 min. The best corresponding kinetic model was determined by fitting the data to the
equations as described in Materials and Methods to obtain correlation coefficients \((R^2)\), and by visual assessment of the Eadie-Hofstee plots (shown as insets in Fig. 4). In human and dog liver microsomal fractions, R3G kinetics exhibited substrate inhibition \((R^2 = 0.90-0.99)\), but the \(K_v\) values were markedly different (human: 689 ± 195 µM; dog: 2900 ± 642 µM). When kinetic profiles for the concentration range of 10–600 µM resveratrol were estimated, data from human liver microsomes best fitted the sigmoidal Hill model \((R^2 = 0.99; n = 2.11 ± 0.11)\), whereas data from dog microsomes followed Michaelis-Menten kinetics \((R^2 = 0.94)\). These differences are clearly recognizable in the corresponding Eadie-Hofstee Plots, which have a shape indicative of auto-activation and linearity in the lower concentration range. In contrast, R3G formation catalyzed by rat and mouse microsomes exhibited a sigmoidal kinetic profile consistent with homotropic activation, as characterized by curved Eadie-Hofstee plots \((R^2 = 0.99, n = 1.80 ± 0.17\) and \(1.64 ± 0.11)\), respectively) \((32)\). Intrinsic clearance values, quantifying the glucuronidation capacity of the enzyme source, were as follows: rat > mouse > dog > human. Table 1 summarizes the kinetic parameters for resveratrol-3-O-glucuronidation.

Species differences in the kinetic profiles of resveratrol-4'-O-glucuronidation: The kinetic profiles for resveratrol-4'-O-glucuronidation are depicted in Figure 5 with their corresponding Eadie-Hofstee plots shown as insets. The respective enzyme kinetic parameters estimated are listed in Table 2. Again, pronounced interspecies differences were observed. While formation of R4'G exhibited classical Michaelis-Menten kinetics \((R^2 = 0.99)\) in human liver microsomes, dog microsomes showed a mixed kinetic profile that was dependent on the concentration range. At lower resveratrol concentrations, the data fitted a hyperbolic Michaelis-Menten model \((R^2 = 0.99)\), but substrate inhibition was observed when the concentration exceeded 600 µM \((K_i: 2000 ± 252 µM)\). This mixed profile is also evident in the corresponding Eadie-Hofstee plot that shows linearity in the lower concentration range and a characteristic hook in the upper quadrant \((27)\). In contrast, R4'G-formation in rat and mouse microsomes again exhibited sigmoidal Hill kinetics \((rat: n = 1.74 ± 0.11, R^2 = 0.99; mouse: n = 2.25 ± 0.19, R^2 = 0.99)\), resulting in typically hooked Eadie-Hofstee plots. Intrinsic clearance values \((V_{max}/K_m)\) of R4'G formation were as follows: human > dog > mouse > rat.
The formation of resveratrol glucuronides by recombinant human UGTs of family 1 and 2 was screened using 50 µM resveratrol. As can be seen in Figure 6, formation of both R3G and R4G is mainly catalyzed by UGT1A1 (41.3 ± 3.7 and 32.9 ±
Data are expressed as estimate ± SE, n = 3–6.

*Kinetic parameters were calculated using Eq. (1) (10–2000 µM (human), 10–200 µM (UGT1A1), and 10–600 µM (dog) resveratrol).

Kinetic parameters were calculated using Eq. (2) (10–2000 µM resveratrol).

Kinetic parameters were calculated using Eq. (3) (10–2000 µM resveratrol).

n.a. = not applicable.

Table 2. Kinetic parameters of the formation of resveratrol-4′-O-glucuronide by human, rat, mouse, and dog liver microsomes and recombinant UGT 1A1 and 1A9

<table>
<thead>
<tr>
<th>Species</th>
<th>Model</th>
<th>( V_{\text{max}} ) (nmol/mg.min)</th>
<th>( K_{\text{m}} ) (µM)</th>
<th>( V_{\text{max}}/K_{\text{m}} ) (ml/mg.min)</th>
<th>n</th>
<th>( K_{\text{i}} ) (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>Michaelis-Menten (^a)</td>
<td>1.28 ± 0.04</td>
<td>250 ± 22.8</td>
<td>5.21 ± 0.74</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td></td>
<td>Michaelis-Menten (^b)</td>
<td>0.21 ± 0.02</td>
<td>46.5 ± 10.5</td>
<td>4.52 ± 0.64</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>UGT1A1</td>
<td>Substrate inhibition (^b)</td>
<td>0.32 ± 0.04</td>
<td>87.3 ± 18.8</td>
<td>3.98 ± 0.98</td>
<td>n.a.</td>
<td>349 ± 69.1</td>
</tr>
<tr>
<td>UGT1A9</td>
<td>Hill (^c)</td>
<td>2.79 ± 0.07</td>
<td>370 ± 25.6</td>
<td>7.52 ± 0.25</td>
<td>2.12 ± 0.17</td>
<td>n.a.</td>
</tr>
<tr>
<td>Dog</td>
<td>Michaelis-Menten (^c)</td>
<td>1.03 ± 0.02</td>
<td>235 ± 10.2</td>
<td>4.33 ± 0.29</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>Rat</td>
<td>Substrate inhibition (^b)</td>
<td>1.24 ± 0.06</td>
<td>299 ± 25.9</td>
<td>4.19 ± 0.43</td>
<td>n.a.</td>
<td>2000 ± 252</td>
</tr>
<tr>
<td>Mouse</td>
<td>Hill (^c)</td>
<td>0.10 ± 0.01</td>
<td>450 ± 24.5</td>
<td>0.23 ± 0.02</td>
<td>1.74 ± 0.11</td>
<td>n.a.</td>
</tr>
</tbody>
</table>

2.8 pmol/mg.min, respectively) and UGT1A9 (98.7 ± 10.4 and 8.7 ± 2.4 pmol/mg.min, respectively). UGT1A6, 1A7, and 1A10 exhibited only low catalytic activities, whereas 1A3, 1A4, 2B7, and 2B15 were inactive. Consequently, the kinetic profiles of resveratrol glucuronidation by UGT1A1 and UGT1A9 were evaluated with special attention paid to the lower substrate concentration ranges (Fig. 7). Formation of R3G in both UGT1A1 and 1A9 exhibited substrate inhibition \((K_{\text{i}} = \text{UGT1A1: } 826 ± 346 \text{ µM;} \text{ UGT1A9: } 640 ± 124 \text{ µM})\). However, when kinetic profiles for the concentration range from 10–600 µM resveratrol were estimated, data from UGT1A1 best fitted the sigmoidal Hill model (n = 2.03 ± 0.11), whereas data from UGT1A9 followed classical Michaelis-Menten kinetics. R4′G formation catalyzed by UGT1A1 also exhibited substrate inhibition \((K_{\text{i}} = 349 ± 69.1 \text{ µM})\) with Michaelis-Menten kinetics before the onset of inhibition. Interestingly, for UGT1A9, we observed pronounced auto-activation (0.99; n = 2.12 ± 0.17, 1). Intrinsic clearance values (see Tables 1 and 2) indicate that UGT1A1 and 1A9 show comparable catalyzing activity for R3G formation \((8.94 ± 0.22 \text{ and } 9.28 ± 0.41 \text{ ml/mg.min, respectively})\), while R4′G formation via UGT1A9 is about twice that via UGT1A1 \((7.52 ± 0.25 \text{ to } 3.98 ± 0.88 \text{ ml/mg.min})\).

Discussion

Resveratrol, a phytoestrogen present in the skin of grapes and other plants, foods and wine, provides a broad spectrum of pharmacological and therapeutic health benefits. Pharmacological studies of resveratrol have been conducted in various animal models and humans; however, species-dependent variations in biotransformation are known to lead to significant differences in quantity and quality of biotransformation products. To obtain reliable extrapolation between animal models and humans, animals that display similar metabolic patterns to humans should be used in these studies. So far, there are no data available about species-dependent variations in resveratrol glucuronidation. Thus, in the present study, we compared for the first time the formation of resveratrol glucuronides in human, dog, rat, and mouse liver microsomes to investigate species differences in resveratrol metabolism.

When we quantified the amount of resveratrol that is transformed into R3G and R4′G by the respective species, we found that rat and mouse liver microsomes have a significantly higher glucuronidating activity than human and dog microsomes (90% of applied dose vs. 65% of applied dose, respectively). Interestingly, a tenfold increase of resveratrol concentration considerably impaired the glucuronide formation in human and dog microsomes (only 10% of
the applied dose was metabolized), but led to only a slight impairment in mouse and no impairment in rat liver microsomes. Distinct species-dependent differences also occurred in the stereoselectivity of resveratrol glucuronidation. While in human and dog liver microsomes, R3G and R4'G were generated at an average ratio of 1:5, the formation of R4'G in both rodent species was insignificant (1:45 in mouse; 1:500 in rat). This is in accordance with literature data, reporting that the formation of R3G but not of R4'G was observed in rat and mouse models.13,20 Species-dependent differences were also seen in the kinetic profiles of resveratrol glucuronidation. In all species tested, only the formation of R3G and R4'G by dog microsomes showed an enzyme kinetic profile similar to that of human microsomes. In contrast to human and dog, both rodent species exhibited pronounced auto-activation kinetics.

A comparable species discrepancy in glucuronidation is known for morphine. In humans, morphine is glucuronidated to the inactive morphine-3-O-glucuronide (M3G), which has been suggested to have effects opposite to those of morphine (anti-analgesic and excitatory), and to the highly analgesic 6-O-glucuronide (M6G) at an average ratio of 5.5:1. In contrast, the ratio of M3G to M6G in liver microsomes of mice and rats were 300:1 and 90:1, respectively.30 Because the efficacy of morphine is impacted by the ratio of glucuronide formation, this may also apply to resveratrol. Whether the resveratrol glucuronides have different degrees of biological activity is not yet known; however, recent data has shown that R3S and R4'S do indeed exhibit distinct differences in their biological activities.

The species differences in morphine glucuronidation are caused by different UGT activities: the formation of M3G and M6G in humans is mainly catalyzed by UGT2B7, whereas UGT2b1, the analogous isoform in rats, only forms M3G.32 UGT1A1, which is found mainly in human liver and intestine, is responsible for the 3-O-glucuronidation of bilirubin, estradiol, and drugs such as...
irinotecan, while UGT1A9, which is distributed in many tissues (liver, intestine, breast, kidney, skin), preferentially catalyzes the glucuronidation of bulky phenols, i.e., estrogens, thyroid hormones, flavonoids, and non-steroidal anti-inflammatory drugs (NSAIDs). UGT1A10 is found exclusively in the gastrointestinal tract and contributes significantly to the metabolism of dietary flavonoids. UGT1A6 is expressed in many tissues, e.g., liver, brain, colon, lung, and kidney, and glucuronidates small planar phenols and primary aromaticamines. UGT1A7 is also an extrahepatic isofrom; it is expressed in pancreas, lung, esophagus and stomach and catalyses the glucuronidation of phenols, coumarins, and steroid hormones.\(^{35-39}\)

Intrinsic clearance values calculated from our data indicate that UGT1A1 and 1A9 equally catalyze the formation of R3G but that UGT1A9 is more active in R4’G formation than UGT1A1. R3G formation exhibited substrate inhibition in both human UGT isoforms, whereas formation of R4’G exhibited substrate inhibition in UGT1A1 and Hill kinetics in UGT1A9. Both 1A1 and 1A9 expression in humans show high inter-individual variability due to genetic and environmental factors, which may also have a strong impact on resveratrol glucuronidation.\(^{38}\) Indeed, after the consumption of 300 ml of red wine, resveratrol and its conjugates were up to 540 ng/ml.\(^{40}\) In our study, however, we could not observe any significant inter-individual differences in mouse, rat, dog, and human liver microsomes. This might be explained by the small sample size (n = 5) and the use of pooled animal microsomes. However, in a previous report using isolated perfused rat liver, we found high inter-individual differences in formation rates of resveratrol metabolites.\(^{22}\) High inter-individual variability has also been observed in the organ distribution of resveratrol in mice. Three hours after oral administration of 50 mg/kg \(^{14}\)C]-resveratrol, highest levels of radioactivity were found in the duodenum, followed by kidney, lung, and liver.\(^{41}\) Substantial radioactivity was present in colon and spleen, whereas moderate radioactivity was present in heart, testes, and brain.

The pronounced differences in our study between human protein sources and rodent liver microsomes indicate that resveratrol glucuronidation is catalyzed by different UGT isoforms. Indeed, Ugt1a1 and 1a9 are highly expressed in rat and mouse liver. However, while Ugt1a1 and 1a9 in mouse liver are functionally active, rat Ugt1a9 is a nonfunctional pseudogene and can therefore not contribute to resveratrol glucuronidation.\(^{42-44}\)

In conclusion, our data show that the extent and kinetic profiles of resveratrol glucuronidation exhibit a broad variability depending on substrate concentration and species. Based on our results, we recommend the use of dog rather than mouse or rat as a model for resveratrol glucuronidation.

References


