JPP Journal of Pharmacy and Pharmacology

JPP 2006, 58: 1443–1450

Received May 1, 2006
Accepted July 24, 2006
DOI 10.1211/jpp.58.11.0004
ISSN 0022-3573

Pharmacokinetics of selected stilbenes: rhapontigenin, piceatannol and pinosylvin in rats

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Abstract

The pharmacokinetics of piceatannol, pinosylvin and rhapontigenin were characterized in male Sprague-Dawley rats after single intravenous doses of 10 mg kg^{-1} of each stilbene. Serial blood samples were collected via a catheter inserted into the right jugular vein and plasma samples were analysed for the selected stilbenes concentrations using reverse phase HPLC methods. After an acute intravenous dose of piceatannol, plasma AUC, urine $t_{\frac{1}{2}}$, CL and V_d were 8.48 ± 2.48 μg h mL^{-1}, 19.88 ± 5.66 h, 2.13 ± 0.92 L h^{-1} kg^{-1} and 10.76 ± 2.88 L kg^{-1} (mean ± s.e.m.), respectively. The acute intravenous dose of pinosylvin yielded the plasma AUC, urine $t_{\frac{1}{2}}$, CL and V_d values of 5.23 ± 1.20 μg h mL^{-1}, 13.13 ± 2.05 h, 1.84 ± 0.44 L h^{-1} kg^{-1} and 2.29 ± 0.56 L kg^{-1} (mean ± s.e.m.), respectively. Rhapontigenin intravenous dosing yielded the plasma AUC, urine $t_{\frac{1}{2}}$, CL and V_d values of 8.39 ± 0.10 μg h mL^{-1}, 25.31 ± 1.46 h, 1.18 ± 0.035 L h^{-1} kg^{-1} and 11.05 ± 0.17 L kg^{-1} (mean ± s.e.m.), respectively. Each stilbene was extensively glucuronidated. These stilbenes were predominantly eliminated via non-urinary routes. All three stilbenes were highly distributed into tissues and were highly extracted by the liver. The detectable plasma half-lives of these xenobiotics appear to be relatively short. However, utilizing urinary concentration–time data, much longer elimination half-lives were evident. The estimates of oral bioavailability characterize these stilbenes as poorly bioavailable compounds.

Introduction

Stilbenes are small molecular weight (200–400 g mol^{-1}), naturally occurring compounds found in a wide range of plant sources and fruits, aromatherapy products and dietary supplements. These molecules are synthesized via the phenylpropanoid pathway (Kodan et al 2002). The phenylalanine structure from phenolic compounds is transformed to cinnamate by the enzyme phenylalanine ammonia-lyase (PAL). An acetyl-CoA group is then added by CoA ligase enzyme to yield cinnamoyl-CoA. Lastly, this product is transformed by stilbene synthase to yield a general stilbenoid structure (Table 1). Stilbenoid compounds can be either constitutive or confined to the wood pulp of the host. Stilbenes are also induced in response to environmental stressors (Roupe et al 2006).

Induction of stilbene synthesis and secretion occurs in the fruit or leaves of its host. Stilbenes that have been induced are often referred to as phytoalexins, due to their protective role upon secretion (Bavaresco et al 1999). Stilbenes act as protective agents to defend the plant against viral and microbial attack, excessive ultraviolet exposure and disease (Jeandet et al 1995). Upon environmental threat, the plant host activates the phenylpropanoid pathway and stilbene structures are produced and secreted. Which specific stilbene is produced depends largely on its host, the region of origin and the environmental stimuli (Bavaresco 2003).

There are many stilbenes that have been characterized for pharmacological activity with resveratrol being the most widely studied, having anti-cancer, anti-inflammatory and anti-oxidant activity. Found primarily in the skins of grapes, resveratrol is synthesized in *Vitis vinifera* grapevines in response to fungal infection or other environmental stressors (Fremont 2000).

Piceatannol (trans-3,4,3′,5′-tetrahydroxystilbene), pinosylvin (trans-3,5′-dihdroxystilbene) and rhapontigenin (trans-3,3′,5′-trihydroxy-4′methoxystilbene) are stilbenes that are structurally similar to resveratrol and possess pharmacological activity across many anti-cancer,

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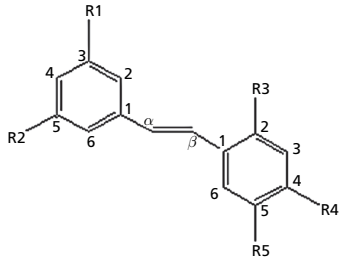
Funding: These studies were funded in part by grants from the Organic Center and an American Cancer Society Institutional Research Grant.

Table 1 Structures of stilbenes



Compound	R1	R2	R3	R4	R5
Piceatannol	OH	OH	H	OH	OH
Pinosylvin	OH	OH	H	H	H
Rhapontigenin	OH	OH	H	OCH_3	OH
Resveratrol	OH	OH	H	OH	H

anti-inflammatory and anti-oxidant assays (Fremont 2000; Roberti et al 2003; Larossa et al 2004; Park et al 2004). It is evident that hydroxyl and methoxy moieties attached to the general stilbene structure produce these varying degrees of activity. Moreover, minute differences in chemical structure may have the capacity to significantly change the metabolism and the pharmacokinetic parameters of these compounds (Sale et al 2004).

Considerable research demonstrating resveratrol to be an attractive candidate in combating a wide variety of cancers and diseases has fuelled interest in characterizing structurally similar stilbene compounds (Sale et al 2004). These stilbene compounds are promising candidates in therapeutic development due to their apparent low toxicity, anti-cancer and anti-inflammatory activity (Fremont 2000). Despite encouraging pharmacological research, there is a paucity of pharmacokinetic data on the disposition of other stilbenes, including piceatannol, pinosylvin and rhapontigenin. Understanding the disposition of these xenobiotics may elucidate concentration–effect relationships and will assist in determining the feasibility of their further pharmaceutical development. We have recently validated novel sensitive and specific chromatographic assays to quantify piceatannol, pinosylvin and rhapontigenin in biological matrices (Roupe et al 2004, 2005a, b). Therefore, the specific objectives of this project are to characterize for the first time the pharmacokinetic disposition of piceatannol, pinosylvin and rhapontigenin in a rat model.

Materials and Methods

Materials

Piceatannol, 4-methylumbelliferone, tetra ethyl ammonium acetate, 7-ethoxycourmarin, daidzein, rhaponticin, β-glucosidase, β-glucuronidase and halothane were purchased from Sigma (St Louis, MO). HPLC-grade acetonitrile, phosphoric acid, methanol and water were purchased from J. T. Baker (Phillipsburg, NJ). Pinosylvin was purchased from Sequoia Research Products Ltd (Oxford, UK). Silastic Laboratory Tubing was purchased from Dow Corning Corporation (Midland, MI). Rhapontigenin was enzymatically synthesized from rhaponticin as described in detail previously (Roupe et al 2005b). Solid phase extraction (SPE) C-18 columns were purchased from Cayman Chemical Company (Ann Arbor, MI). Dulbecco's Modified Eagle Medium (D-MEM) and RMPI 1640 medium were purchased from Gibco Industries Inc. (Langley, OK). Fetal bovine plasma (FBS) was purchased form Equitech-Bio Inc. (Kerrville, TX). Intramedic polyethylene tubing was purchased from Becton Dickinson Primary Care Diagnostica, Becton Dickinson and Company (Sparks, MD). Monoject 23 gauge (0.6 mm × 25 mm) polypropylene hub hypodermic needles were purchased from Sherwood Medical (St Louis, MO). Undyed braided-coated polyglycolic acid suture, synthetic absorbable surgical suture, was purchased from Surgical Specialties Corporation (Reading, PA).

Animals and surgical procedures

Male Sprague-Dawley rats, 340–360 g, were obtained from Simonsen Labs (Gilroy, CA) and given free access to food (Purina Rat Chow 5001) and water in our animal facility for at least 3 days before use. Rats were housed in temperature-controlled rooms with a 12-h light–dark cycle. The day before the pharmacokinetic experiment the right jugular veins of the rats were catheterized with sterile silastic cannula (Dow Corning, Midland, MI) under halothane anaesthesia. This involved exposure of the vessel before cannula insertion. After cannulation, the Intramedic PE-50 polyethylene tubing (Becton, Dickinson and Company, Franklin Lakes, NJ) connected to the cannula was exteriorized through the dorsal skin. The cannula was flushed with 0.9% saline. The rats were transferred to metabolic cages and were fasted overnight. Animal ethics approval was obtained from The Institutional Animal Care and Use Committee at Washington State University.

Experimental design

Fifteen male Sprague Dawley rats, average weight 350 g, were cannulated as described in the previous section. Each of the rats were placed in separate metabolic cages, allowed to recover overnight and fasted for 12 h before dosing. On the day of experiment, the rats were dosed either intravenously with piceatannol ($10\,mg\,kg^{-1}$), pinosylvin ($10\,mg\,kg^{-1}$) or rhapontigenin ($10\,mg\,kg^{-1}$) dissolved in PEG-400 ($n=5$ for each treatment group). Serial blood samples (0.25 mL) were collected at 0, 1 and 10 min, and 0.5, 1, 2, 4, 6, 12 and 24 h. After each sample collection, the cannulas were flushed with 0.25 mL of saline. Following centrifugation of the blood samples, plasma was collected and stored at −70°C until analysed. Urine samples were also collected in metabolic urine cups at 0, 2, 6, 12, 24, 24, 48, 72 and 84 h following stilbene administration and were stored at −70°C until analysed.

HPLC chromatography

The analysis of piceatannol was carried out using a Shimadzu HPLC (Kyoto, Japan), consisting of an LC-10AD pump, a SIL-10A auto injector, an RF-535 fluorescence detector, an

SPD-M10A UV/VIS spectrophotometric detector and an SCL-10A system controller. Data collection and integration were accomplished using a Shimadzu CR501 chromatopac integrator. The analytical column used was a Phenomenex C18 (250×4.6 mm, i.d. 5 μm) (Torrance, CA) equipped with a Phenomenex C18 (4×3.0 mm, i.d. 5 μm) guard column. The mobile phase consisted of methanol–0.04% phosphoric acid (34:66, v/v), filtered and degassed under reduced pressure before use. Separation was carried out isocratically at ambient temperature, and a flow rate of 1 mL min^{-1}, with fluorescence detection excitation of 320 nm and emission at 420 nm (Roupe et al 2004). Pinosylvin pharmacokinetic samples were analysed using a Shimadzu HPLC (Kyoto, Japan), consisting of an LC-10AT pump, a SIL-10AF auto injector, a photodiode-array SPD-10A *VP* UV/VIS spectrophotometric detector and an SCL-10A system controller. The injection volume was 50 μL. Data collection and integration were accomplished using Shimadzu EZ start 7.1.1 program software. The analytical column used was an amylose tris 3,5-dimethylphenylcarbamate (150×4.6 mm, i.d. 5 μm) (Chiral Technologies Inc. Exton, PA). The mobile phase consisted of acetonitrile–0.1% phosphoric acid (42:58, v/v), filtered and degassed under reduced pressure before use. Separation was carried out isocratically at ambient temperature, and a flow rate of 0.500 mL min^{-1}, with UV detection at 308 nm (Roupe et al 2005a).

For the analysis of rhapontigenin, the HPLC system used was a Shimadzu HPLC (Kyoto, Japan), consisting of an LC-10AT pump, a SIL-10AF auto injector, a photodiode-array SPD-10A *VP* UV/VIS spectrophotometric detector and an SCL-10A system controller. Injection volume was 150 μL. Data collection and integration were accomplished using Shimadzu EZ start 7.1.1 program software. The analytical column used was an amylose tris 3,5-dimethylphenylcarbamate (150×4.6 mm, i.d. 5 μm) (Chiral Technologies Inc. Exton, PA). The mobile phase consisted of acetonitrile–0.1% phosphoric acid (30:70, v/v), filtered and degassed under reduced pressure before use. Separation was carried out isocratically at ambient temperature, and a flow rate of 1.00 mL min^{-1} with UV detection at 324 nm (Roupe et al 2005b).

Mass spectrometry

Samples were applied to an API 4000 triple quadrupole mass spectrometer (Applied Biosystems Sciex, ON) using negative ion electrospray under similar chromatographic conditions to those mentioned above with the exceptions that an Agilent 1100 series HPLC system (Palo Alto, CA) was employed, consisting of: autosampler, binary pump, degasser and UV detector and phosphoric acid was omitted as a modifier, while 1 mM $(NH_4)HCO_3$ was used to maintain neutral pH. The mass spectrometer was operated under conditions optimized for rhapontigenin at the chromatographic flow conditions (0.5 mL min^{-1}) as follows: the Ionspray needle was maintained at –4500 kV, with nitrogen as drying gas 1 (setting 40), drying gas 2 (setting 25), curtain gas (setting 10) and collision gas (setting 4). The turbospray interface was maintained at 400°C. The declustering potential (DP), collision energy (CE) and exit potential (EP) were optimum at 30 V, 45 eV and 10 V, respectively. Both the Q1 and Q3 quadrupoles were maintained at unit resolution (0.7 Da width at half height). The characteristic fragmentation reactions include: m/z 212.2→193.2 for pinosylvin, m/z 387.2→212.2 for pinosylvin glucuronide, parent resveratrol m/z 243.2→235.2, m/z 419→242.2 for piceatannol glucuronide, m/z 238→219 for rhapontigenin and m/z 413.2→238 for rhapontigenin glucuronide as previously reported (Roupe et al 2004, 2005a, b).

Pharmacokinetic analysis

Pharmacokinetic analysis was performed using WinNonlin software (Ver. 1). Summary data were expressed as mean ± standard error of the mean (s.e.m.). The elimination rate constant (λ_n) was estimated by linear regression of the plasma concentrations in the log-linear terminal phase. The $AUC_{0-\infty}$ was calculated using the combined log-linear trapezoidal rule for data from time of dosing to the last measured concentration, plus the quotient of the last measured concentration divided by λ_n. Non-compartmental pharmacokinetic methods were used to calculate clearance (CL) and volume of distribution (V_d) after intravenous dosing. The mean hepatic plasma flow in rat is ~1.74 L h^{-1} kg^{-1} (Davies & Morris 1993; Mahboudian-Esfahani & Brocks 1999). Hepatic clearance was determined using equation 1:

$$CL_h = CL_{tot} - CL_r \quad (1)$$

The plasma half-life was determined using equation 2:

$$t_{\frac{1}{2}} = 0.693/\lambda n \quad (2)$$

To determine the fraction of unchanged piceatannol excreted (fe) in urine, the total amount in urine was divided by the total dose administered. The renal clearance (CL_r) was determined by equation 3:

$$CL_r = fe \times CL_{tot} \quad (3)$$

Maximum oral bioavailability is estimated using equation 4:

$$\text{Hepatic CL (L } h^{-1} \text{ } kg^{-1}) = \text{mean hepatic blood flow rate (Q) (L } h^{-1} \text{ } kg^{-1}) \times \text{extraction ratio (ER)} \quad (4)$$

Results

Piceatannol pharmacokinetics

Following intravenous administration of piceatannol a single metabolite was evident in plasma (Figure 1A). The metabolite was determined to be a glucuronidated metabolite, which was verified by incubating the plasma with β-glucuronidase for 2 h, analysing it once again via HPLC and verifying by mass spectrometry.

A small increase in piceatannol concentration was also evident at 6 h indicating the possibility of enterohepatic recycling (Figure 1A). However, this concentration range (< 0.1 μg mL^{-1}) was below the limit of quantification of the assay and is therefore not possible to quantify reliably and was subsequently not included in the pharmacokinetic analysis. The total plasma clearance of piceatannol was

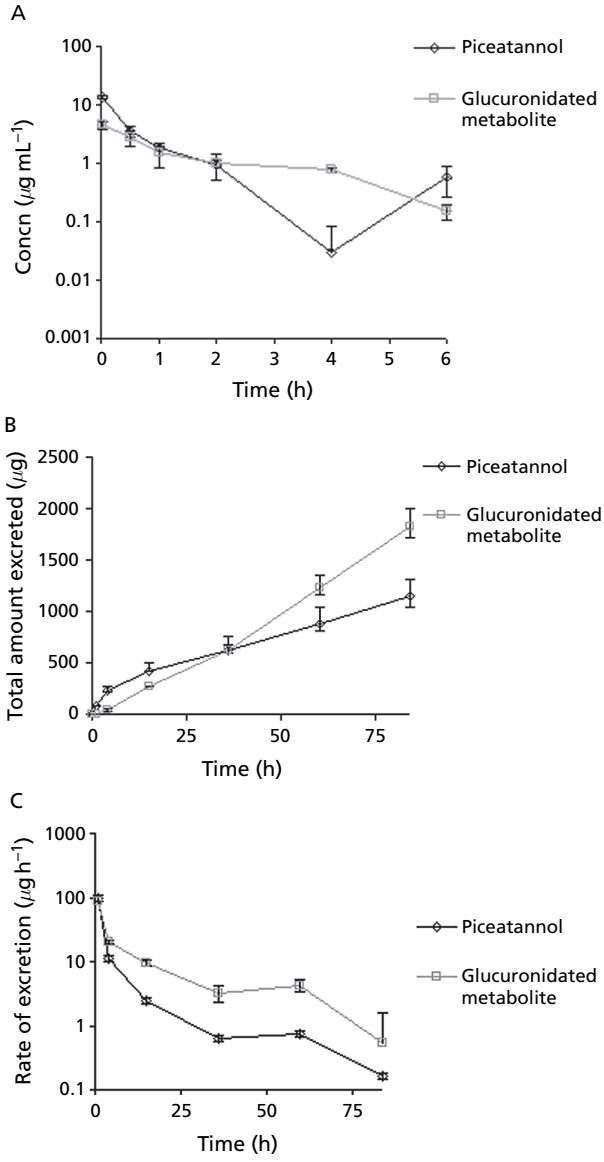


Figure 1 A. Piceatannol disposition in plasma following intravenous administration. Data are means ± s.e.m., n = 5. B. Cumulative piceatannol and glucuronidated metabolite (μg) excreted in urine over 84 h. Data are means ± s.e.m., n = 5. C. Rate of excretion (μg h^{-1}) of piceatannol and glucuronidated metabolite in urine over 84 h. Data are means ± s.e.m., n = 5.

determined to be 2.13 ± 0.92 L h^{-1} kg^{-1}, thus hepatic clearance was $CL_h = 2.13 - 0.699 = 1.43$ L h^{-1} kg^{-1} (Table 2).

The CL_h approaches the mean hepatic plasma flow (~1.74 L $h^{-1}kg^{-1}$) indicating that piceatannol is a high extraction compound and is cleared predominantly via hepatic elimination. The volume of distribution of piceatannol is 10.76 ± 2.88 L kg^{-1}, which is greater than total body water, suggesting piceatannol is highly distributed in tissue. The plasma concentrations of piceatannol appeared to decline rapidly with a mean elimination half-life of 4.23 ± 1.25 h. The mean area under the curve (AUC), representing the total amount of drug exposure in the plasma over time, was 8.48 ± 2.48 μg h mL^{-1}.

Table 2 Pharmacokinetic parameters of stilbenes in the rat

Parameter	Piceatannol	Pinosylvin	Rhapontigenin
AUC_{inf} (μg h mL^{-1})	8.48 ± 2.48	5.23 ± 1.20	8.39 ± 0.10
V_d (L kg^{-1})	10.76 ± 2.88	2.29 ± 0.56	11.05 ± 0.17
Cl_{tot} (L h^{-1} kg^{-1})	2.130 ± 0.920	1.840 ± 0.435	1.180 ± 0.035
CL_h (L h^{-1} kg^{-1})	1.430	1.665	1.165
CL_r (L h^{-1} kg^{-1})	0.669	0.175	0.015

Data are presented as means ± s.e.m.

Urine samples were taken up to 84 h post piceatannol administration and analysed via HPLC. Results revealed that parent piceatannol was detected in the urine 84 h post-dose. The urinary data was modelled using WinNonlin to estimate the half-life of piceatannol in urine. The half-life was determined to be 19.88 ± 5.66 h. The discrepancy of the plasma half-life (4.23 ± 1.25 h) and the urinary half-life (19.88 ± 5.66 h) suggests that the plasma half-life is likely underestimating the overall half-life of piceatannol due to assay sensitivity limits. The glucuronidated metabolite previously identified in plasma was also detected in urine samples (Figure 1B, C). The total dose of piceatannol administered was 10 mg kg^{-1}. The average weight of the rats in this experiment was ~350 g. The administered dose to each rat was ~3.5 mg of piceatannol. The total amount excreted (μg) plot shows that piceatannol excreted in urine (~1149.92 μg) is very small compared with the overall dose administered (3.5 mg). This suggests that piceatannol is eliminated predominately via non-renal excretion. The fraction excreted unchanged fe = 1149.92/3500 × 100 = 32.8%, and therefore CL_r = 0.328 × 2.13 = 0.669 (L h^{-1} kg^{-1}).

Urinary excretion therefore was determined to account for approximately 32.8% total unchanged piceatannol excreted, suggesting that non-renal excretion mechanisms predominate (67.2% of total piceatannol excreted).

Pinosylvin pharmacokinetics

Following intravenous administration of pinosylvin, one metabolite was evident in plasma (Figure 2A). The metabolite was determined to be a glucuronidated metabolite and was verified by incubating the plasma with β-glucuronidase for 2 h and analysing it once again via HPLC and mass spectrometry.

The total plasma clearance of pinosylvin was determined to be 1.84 ± 0.44 L h^{-1} kg^{-1} (Table 2). Hepatic clearance was determined to be $CL_h = 1.84 - 0.18 = 1.67$ L h^{-1} kg^{-1} and this CL_h approaches the mean hepatic plasma flow also indicating that pinosylvin, like piceatannol, is a high extraction compound and is cleared predominantly via hepatic elimination. The volume of distribution of pinosylvin is 2.29 ± 0.56 L kg^{-1}, suggesting that pinosylvin is highly distributed in tissue. The plasma concentrations of pinosylvin appeared to decline rapidly with a mean elimination half-life of 0.82 ± 0.05 h. The plasma half-life was determined to be: $t\frac{1}{2} = 0.693/\lambda_h = 0.82 \pm 0.05$ h. The mean area under the curve (AUC), representing the total amount of drug exposure in the plasma over time, was 5.23 ± 1.20 μg h mL^{-1}.

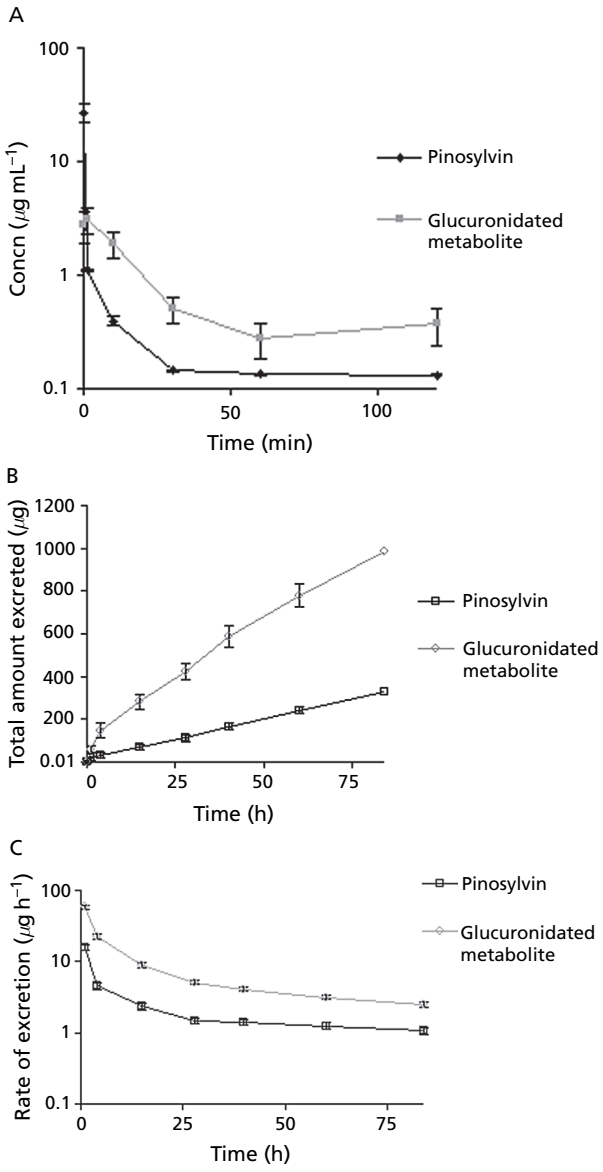


Figure 2 A. Pinosylvin disposition in plasma following intravenous administration. Data are means ± s.e.m., n = 5. B. Cumulative pinosylvin and glucuronidated metabolite (μg) excreted in urine over 84 h. Data are means ± s.e.m., n = 5. C. Rate of excretion (μg h^{-1}) of pinosylvin and glucuronidated metabolite in urine over 84 h. Data are means ± s.e.m., n = 5.

Parent pinosylvin was detected in the urine 84 h post dose. The urinary data was modeled using WinNonlin to estimate the half-life of pinosylvin in urine. The half-life was determined to be 13.13 ± 2.05 h. The discrepancy of the plasma half-life (0.82 ± 0.05 h) and the urinary half-life (13.13 ± 2.05 h) illustrates that the plasma half-life is likely underestimating the overall terminal elimination half-life of pinosylvin. The glucuronidated metabolite previously identified in plasma pharmacokinetic samples was also detected in urine samples (Figure 2B, C). The amount remaining to be excreted (μg) (ARE) plot shows that pinosylvin excreted in the urine (~331.6 μg) is small compared with the overall dose administered (3.5 mg). This suggests that, like resveratrol and piceatannol, pinosylvin is eliminated predominantly via non-renal excretion. To determine the fraction of unchanged piceatannol excreted (fe) in urine, the total amount in urine was divided by the total dose administered $fe = 331/3500 \times 100 = 9.46\%$. The renal clearance ($CL_r$) was determined to be $CL_r = 0.095 \times 1.84 = 0.175$ (L h^{-1} kg^{-1})

Urinary excretion therefore was determined to account for approximately 9.46% of total pinosylvin excreted, indicating that non-renal excretion predominates (90.54% of total pinosylvin excreted).

Rhapontigenin pharmacokinetics

Following intravenous administration of rhapontigenin, one predominant metabolite was present in plasma (Figure 3A). The metabolite was determined to be a glucuronidated metabolite and was verified by incubating the plasma with β-glucuronidase for 2 h, analysing it once again via HPLC and also verifying with mass spectrometry.

The total plasma clearance of rhapontigenin was determined to be 1.18 ± 0.87 L h^{-1} kg^{-1}. Hepatic clearance was determined to be $CL_h = 1.18 - 0.015 = 1.165$ L h^{-1} kg^{-1}, a value that approaches the mean hepatic plasma flow, suggesting that rhapontigenin is a high extraction compound and is cleared predominantly via hepatic elimination. The volume of distribution of rhapontigenin is 11.05 ± 0.17 L kg^{-1}, consistent with rhapontigenin being highly distributed to tissue. The plasma concentrations of rhapontigenin declined rapidly and the plasma half-life was determined to be $t_{\frac{1}{2}} = 0.693/\lambda_n = 3.0 \pm 1.35$ h. The mean area under the curve (AUC) was 8.39 ± 0.10 μg h mL^{-1}.

Urine samples were taken up to 84 h post rhapontigenin administration and analysed via HPLC. Results revealed that, like piceatannol and pinosylvin, rhapontigenin was detected in the urine 84 h post dose (Figures 3B, C). The urinary data was modelled using WinNonlin to estimate the half-life of rhapontigenin in urine. The half-life was determined to be 25.31 ± 1.46 h. The discrepancy of the plasma half-life (3.0 ± 1.35 h) and the urinary half-life (25.31 ± 1.46 h) illustrates that the plasma half-life is underestimating the overall half-life of rhapontigenin. These data also illustrate that the urine half-life is a better estimate of terminal elimination half-life.

The glucuronidated metabolite identified in plasma pharmacokinetic samples was also detected in urine samples. The total amount excreted (μg) plot shows that rhapontigenin excreted in urine (~48.30 μg) is very small compared with the overall dose given (3.5 mg). This indicates that, like piceatannol and pinosylvin, rhapontigenin is eliminated predominantly via non-renal excretion. To determine the fraction of unchanged rhapontigenin excreted (fe) in urine, the total amount in urine was divided by the total dose administered $fe = 48.30/3500 \times 100 = 1.25\%$. The renal clearance ($CL_r$) was determined to be $CL_r = 0.014 \times 1.18 = 0.015$ (L h^{-1} kg^{-1}).

Urinary excretion therefore was determined to account for approximately 1.25% of total rhapontigenin excreted, indicating that non-renal excretion predominates (98.75% of total rhapontigenin excreted).

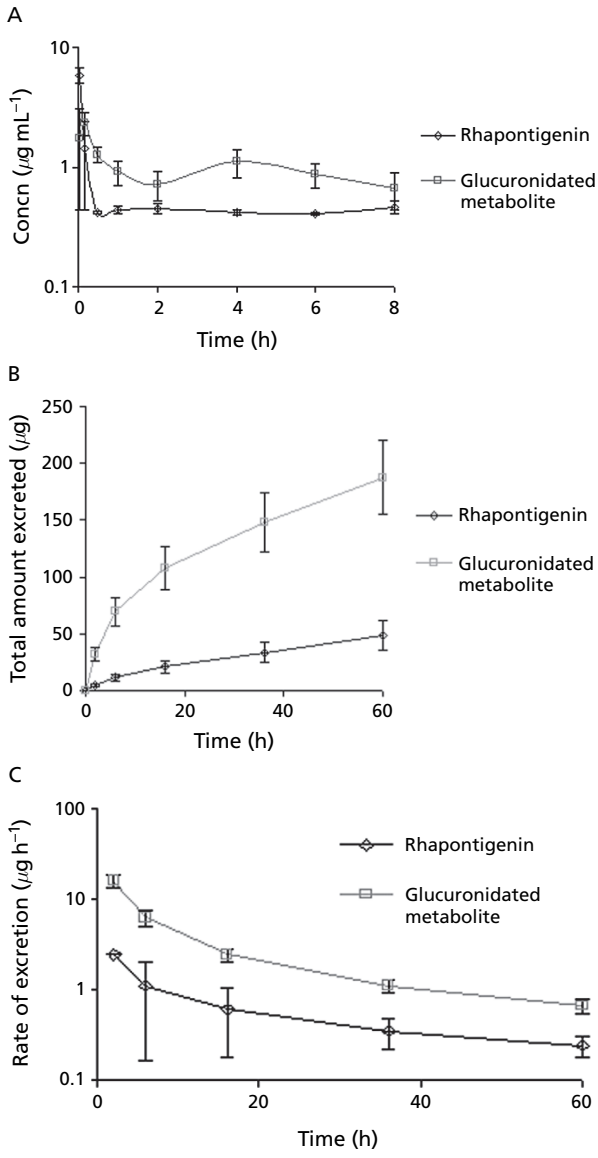


Figure 3 A. Rhapontigenin disposition in plasma following intravenous administration. Data are means ± s.e.m., n = 5. B. Cumulative rhapontigenin and glucuronidated metabolite (μg) excreted in urine over 84 h. Data are means ± s.e.m., n = 5. C. Rate of excretion (μg h^{-1}) of rhapontigenin and glucuronidated metabolite in urine over 84 h. Data are means ± s.e.m., n = 5.

Discussion

All three stilbenes examined (piceatannol, pinosylvin and rhapontigenin) each undergoes extensive glucuronidation upon intravenous administration, as determined by plasma and urine concentrations and later verified treating plasma and urine samples with β-glucuronidase and verified with mass spectrometry. This in-vivo data parallels our in-vitro metabolism findings in rat liver microsomes with each stilbene whereby glucuronidation is rapid and predominates (Roupe et al 2005c, d, e). This is also consistent with previous studies of resveratrol research in rodents and man, showing that resveratrol exists predominantly in its glucuronic acid conjugated forms in plasma and urine (Aseni et al 2002; Juan et al 2002; Marier et al 2002; Goldberg et al 2003; Walle et al 2004; Wang et al 2005; Wenzel & Somoza 2005).

Previous research has also suggested that stilbenes such as resveratrol undergo extensive hepatic clearance (Marier et al 2002; Wenzel & Somoza 2005), where they are metabolized to glucuronidates and sulfate metabolites (Wang et al 2005) and are highly absorbed (Walle et al 2004). Therefore, it reasonable to deduct that non-renal clearance represents the hepatic clearance of these compounds and that the maximum oral bioavailability of these stilbenes are equal to the hepatic extraction ratio. The current findings with rhapontigenin demonstrate that replacement of the phenol functional group with methoxy moieties and addition of a further methoxy group alter pharmacokinetic properties of the parent molecule, demonstrating differential pharmacokinetics (Sale et al 2004). Piceatannol, pinosylvin and rhapontigenin are predominantly eliminated via non-urinary routes, likely via biliary excretion, and to a much lesser extent, via urinary excretion. All three stilbenes, like resveratrol, are lipophilic with partition coefficient (LogP values ~3–4) and are highly distributed into the tissue as evidenced by the high volume of distribution values. In addition, these compounds are highly extracted, as suggested by their clearance values. The liver is the main organ responsible for clearing the selected stilbenes with hepatic clearance values approaching mean hepatic plasma flow.

We utilized two commonly employed plots to estimate the half-life of these stilbenes. The rate of urinary excretion plot (Figures 1C, 2C and 3C) are used to establish how rapidly drug is being eliminated from the body. The main disadvantage of this method is that error can skew the overall plot and interpretation will lack precision. Additionally, this method requires that the investigator collects precisely timed samples, and in working with drugs of short half-lives, this can be especially difficult both on subject and researcher. The other plot commonly employed in determining half-life is the plasma concentration vs time plot. This method allows the investigator to establish important pharmacokinetic parameters such as volume of distribution and clearance. However, the experimental design requires invasive sample collection and can be problematic when researching drugs that are highly distributed to the tissues and are therefore not present in sufficiently high concentrations in the plasma for assay detection over long time periods.

Pharmacokinetic theory suggests that the slopes of the rate of urinary excretion plot (log (dXu/dt) = log (ke X0) − KE/2.303t), and the plasma concentration vs time plot (log C = log Co − KE/2.303t) must be parallel with an identical parallel slope (−KE/2.303). The half-life determined by ($t\frac{1}{2} = 0.693/KE$) should also theoretically be the same. However, with the present data the rate plot is not parallel to the slope of the plasma concentration vs time plots (Figures 1A, C, 2A, C, 3A, C). This suggests that the urinary half-life is a better estimate of true terminal elimination half-life of these stilbenes. The urinary excretion kinetics of resveratrol has not been previously ascertained and should be examined in light of these findings.

The estimates of maximum oral bioavailability characterize these stilbenes as poorly bioavailable compounds. Based on these pharmacokinetic data, piceatannol, pinosylvin and rhapontigenin appear to be circulating to a large extent within the reticuloendothelial system. The lumen of the intestine, as well as the endothelial lining of the liver, is exposed to the free, unchanged fraction of the administered stilbene before its glucuronidated biotransformation and these are likely responsible for their poor bioavailability.

Extremely rapid glucuronidation conjugation by the liver and intestine appears to be the rate-limiting step in bioavailability. Resveratrol is known to undergo metabolic phase II reactions involving conjugation with glucuronic acid and sulfate. This poor bioavailability has been demonstrated in mice, rats and dogs, and collectively and consistently suggested that while well absorbed, there is rapid glucuronidation and sulfation both in the liver and in intestinal epithelial cells (Asensi et al 2002; Juan et al 2002; Marier et al 2002). Several studies in man also hint at a poor bioavailability of resveratrol (Goldberg et al 2003; Walle et al 2004). The pharmacokinetic differences between resveratrol and a resveratrol analogue DMU 212 in concentration achieved in tissues may suggested differential metabolic pathways of stilbene analogues (Sale et al 2004).

Stilbenes are poorly aqueous soluble lipophilic xenobiotics. Formulation scientists have attempted to solve this issue through the use of pro-drugs and other structural synthetic manipulations and modification of stilbene analogues (Pettit et al 2002; Roberti et al 2003; Sale et al 2004; Chen et al 2005), polyethyleneglycol conjugates delivery systems (Zhang et al 2005) and nanoparticle micellular formulations (Yao et al 2006). These structural alterations have been aimed at optimization of the pharmacodynamics and pharmacokinetics. Indeed it has been suggested that such pharmacokinetic exploration should be an essential part of the chemopreventive drug discovery process of stilbenes (Sale et al 2004). Replacement of the phenol functionalities in resveratrol by methoxy moieties and an addition of a further methoxy group have been demonstrated to alter the pharmacokinetic properties of the parent molecule (Sale et al 2004). Additional pharmacokinetic/pharmacodynamic studies need to be designed to help rationalize the choice of resveratrol analogues for further exploratory testing for potential therapeutic usefulness.

Further exploration of the pharmacodynamics of these compounds has demonstrated utility in targeted treatment of gastrointestinal disease such as colitis and colorectal cancer (Roupe 2005; Roupe et al 2005c, d, e). Improvements in bioavailability through formulation optimization may also extend their possible therapeutic uses and further studies are ongoing.

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