In Vitro Inhibition of Rat CYP1A1 and CYP1A2 by Piceatannol, a Hydroxylated Metabolite of *trans*-Resveratrol

Thomas K.H. Chang*, Jie Chen and Chia-Ting Yu

Faculty of Pharmaceutical Sciences, The University of British Columbia, Vancouver, British Columbia, V6T 1Z3, Canada

Abstract: Piceatannol and its parent compound, *trans*-resveratrol, decreased the *in vitro* catalytic activity of rat CYP1A1 and CYP1A2 by mixed inhibition. *trans*-Resveratrol was not a mechanism-based inactivator of rat CYP1A *in vitro* and the administration of this compound (50 mg/kg) to rats did not affect hepatic microsomal CYP1A-mediated enzyme activity.

Key Words: CYP1A1, CYP1A2, cytochrome P450, piceatannol, *trans*-resveratrol.

INTRODUCTION

Root extracts of the weed *Polygonum cuspidatum* has been used in traditional Chinese medicine to treat a variety of diseases [1]. One of the chemical constituents present in this plant is *trans*-resveratrol [2], also known as 3,5,4′-trihydroxy-*trans*-stilbene (Fig. **1**). This polyphenolic compound is also found in other plant genera, including *Vitaceae* [3] and *Arachis* [4]. Other natural sources of *trans*-resveratrol include wine, especially red wine, grapes, and berries [5].

trans-Resveratrol is metabolized mainly by conjugation reactions catalyzed by specific human UDP-glucuronosyltransferases, such as UGT1A1 and UGT1A9 [6,7], and human sulfotransferases, such as SULT1A1 and SULT1E1 [8]. It also undergoes hydroxylation catalyzed by specific human cytochrome P450 (CYP) enzymes, such as CYP1A1, CYP1A2, and CYP1B1, to form piceatannol (Fig. **1**) [9,10], which is also referred to as 3,4,3′,5′,-tetrahydroxy-*trans*-stilbene or 3-hydroxyresveratrol.

trans-Resveratrol decreases the incidence of mammary tumors in rodents administered with 7,12-dimethylbenz[*a*]anthracene (DMBA) [11-13], but the detailed molecular and cellular mechanisms by which this polyphenolic compound inhibits DMBA-induced tumorigenesis is not well-understood. *trans*-Resveratrol may act as a blocking agent at the initiation step of carcinogenesis by inhibiting procarcinogen-bioactivating enzymes because *in vitro* studies have shown that this compound directly inhibits the catalytic activity of DMBA-bioactivating human CYP1A1 [14-17], CYP1A2 [14, 18], and CYP1B1 [19]. *trans*-Resveratrol is also a mechanism-based inactivator of human CYP1A2, but not CYP1A1 [14]. However, it is not known whether *trans*-resveratrol decreases rat CYP1A1 and CYP1A2 catalytic activity by direct inhibition and/or mechanism-based inactivation. It is also not known whether piceatannol, which is an oxidative metabolite of *trans*-resveratrol, differentially inhibits these CYP1A enzymes. Therefore, in the present study, we investigated the *in vitro* effect of *trans*-resveratrol and piceatannol

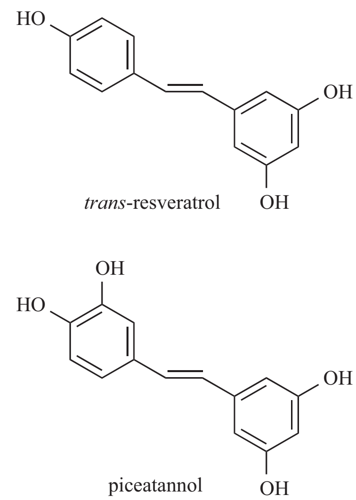

Fig. (1). Chemical structures of *trans*-resveratrol and piceatannol.

on the catalytic activity of rat CYP1A1 and CYP1A2 and determined the effect of *in vivo* administration of *trans*-resveratrol on rat hepatic microsomal CYP1A-mediated enzyme activities.

MATERIALS AND METHODS

Chemicals, Reagents, and cDNA-expressed Enzymes

trans-Resveratrol (99.4% pure, lot no. RES-0301-01) was a gift from Royalmount Pharma (Montreal, Quebec, Canada). Piceatannol, 7-ethoxyresorufin, 7-methoxyresorufin, β-naphthoflavone (BNF), NADPH, and hydroxypropyl γ-cyclodextrin were bought from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Authentic resorufin metabolite standard was obtained from Molecular Probes, Inc. (Eugene, OR, U.S.A.). cDNA-expressed rat CYP1A1 and cDNA-expressed rat CYP1A2 were purchased from BD GENTEST (Woburn, MA, U.S.A.).

Animals

Adult male Sprague-Dawley rats (250-275 g) were purchased from Charles River Co. (Montreal, Quebec, Canada) and acclimatized in our animal care facility for at least 7 days prior to the initiation of treatment. They were housed

*Address correspondence to this author at the Faculty of Pharmaceutical Sciences, The University of British Columbia, 2146 East Mall, Vancouver, BC, V6T 1Z3, Canada; Fax: 1-604-822-3035;
E-mail: tchang@interchange.ubc.ca

1872-3128/07 $50.00+.00

on corn cob bedding in a temperature (22°C) and light (7 a.m. on and 7 p.m. off) controlled room, provided with food (Rodent Laboratory Diet #5002, PMI Feeds, Inc., Richmond, IN) and tap water *ad libitum*, and cared for in accordance with the principles and guidelines of the Canadian Council on Animal Care.

Treatment of Animals and Preparation of Microsomes

In one experiment, rats were treated either orally or i.p. with *trans*-resveratrol (50 mg/kg) or the vehicle (20% hydroxypropyl γ-cyclodextrin / 2% Tween 80 in a ratio of 10:7, v/v) and terminated at 10-60 min after dosing. In other experiments, rats were pretreated i.p. once daily for three consecutive days with BNF (suspended in corn oil) at a dosage of 40 mg/kg. *trans*-Resveratrol (50 mg/kg) or the vehicle (20% hydroxypropyl γ-cyclodextrin / 2% Tween 80 in a ratio of 10:7, v/v) was administered either by oral gavage or i.p. one day after the last dose of BNF. Rats were terminated between 6-90 min after the dose of *trans*-resveratrol. The range of termination times were selected based on the elimination half-life of the compound [20,21]. Livers were excised, rinsed in ice-cold buffer containing 50 mM Tris (pH 7.4) and 1.15% KCl, and used immediately to prepare microsomes [22]. Microsomal protein concentration was determined using the Bio-Rad Protein Assay Kit.

7-Alkoxyresorufin *O*-Dealkylation Assays

7-Ethoxyresorufin *O*-dealkylation (EROD) and 7-methoxyresorufin *O*-dealkyation (MROD) activities were determined by a continuous spectrofluorometric method as described previously [14]. Briefly, each standard 2 ml incubation mixture contained 100 mM potassium phosphate (pH 7.4), 1.5 mM EDTA, 0.25 µM 7-ethoxyresorufin or 7-methoxyresorufin, 0.25 mM NADPH, and rat hepatic microsomes (10 µg microsomal protein for the EROD assay and 30 µg microsomal protein for the MROD assay) or cDNA-expressed rat enzymes (2.5 pmol CYP1A1 or 5 pmol CYP1A2). Calibration curves were constructed with authentic resorufin. Preliminary experiments indicated that the assay was linear with respect to microsomal protein concentration and incubation time.

Enzyme Inhibition and Inactivation Experiments

The *in vitro* enzyme inhibition experiments were performed with varying concentrations of 7-ethoxyresorufin (0.05, 0.1, 0.2, and 0.4 µM), 7-methoxyresorufin (0.1, 0.2, 0.4, and 0.8 µM), piceatannol (0, 0.5, 1, and 2 µM), and *trans*-resveratrol (0, 0.25, 0.5, and 1 µM). The mode of enzyme inhibition and the apparent K_i values were determined by non-linear regression analysis (Enzyme Kinetics Module software program, version 1.1, SPSS Science, Chicago, IL, U.S.A.) as detailed earlier [23]. Enzyme inactivation experiments were performed as described previously [14], except that rat hepatic microsomes (12.5 µg microsomal protein) or cDNA-expressed rat enzymes (6.25 pmol CYP) were in the preincubation mixture.

Statistical Analysis

The difference between the means of two groups was assessed by the two-tailed, independent t-test. The difference between the means of more than two groups was assessed by one-way analysis of variance and, if applicable, was followed by the Student Newman-Keuls multiple comparison test (SigmaStat™ statistical software program, SPSS Science, Chicago, IL, U.S.A.). The level of significance was set *a priori* at $p < 0.05$.

RESULTS AND DISCUSSION

Piceatannol is a CYP1-catalyzed oxidative metabolite of *trans*-resveratrol [9,10] and has anti-proliferative activity *in vitro* [24]. As shown in the present study with cDNA-expressed enzymes, piceatannol and *trans*-resveratrol inhibited the catalytic activity of rat CYP1A1 and CYP1A2 (Fig. **2**). Inhibition of rat hepatic microsomal CYP1A enzymes by these two compounds was confirmed by a concentration-dependent decrease in MROD activity and EROD activity (data not shown). According to immuno-inhibition experiments, rat hepatic microsomal MROD activity reflects pri-

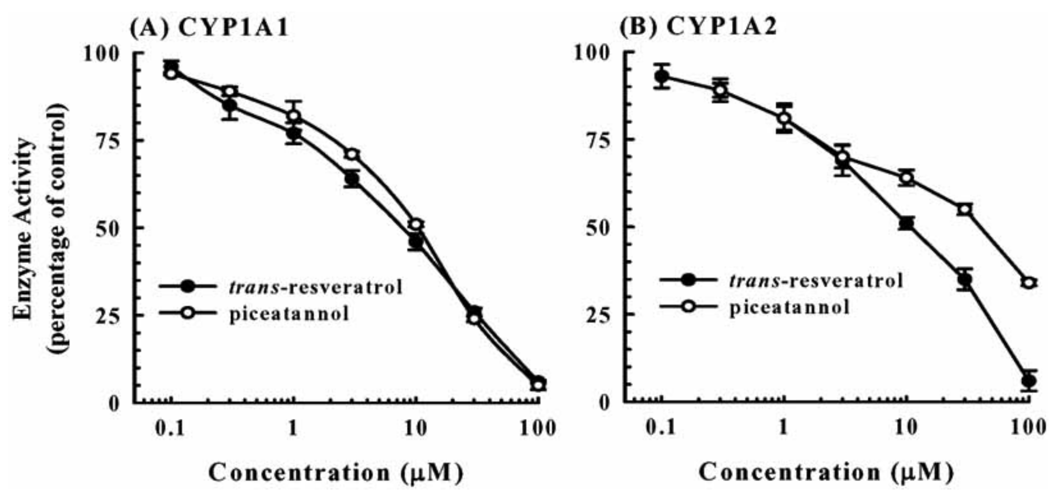

Fig. (2). Dose-response curves for the effect of piceatannol and *trans*-resveratrol on the catalytic activity of rat CYP1A1 and CYP1A2. The EROD assay (0.25 µM substrate) was conducted with cDNA-expressed CYP1A1 (2.5 pmol, panel A) or CYP1A2 (5 pmol, panel B) and varying concentrations (0.1-100 µM) of piceatannol or *trans*-resveratrol. Control incubations contained 0.15% v/v methanol (vehicle). Data are expressed as mean ± SEM percentage of control activity (N = three independent experiments).

marily CYP1A2 rather than CYP1A1 activity, whereas rat hepatic microsomal EROD activity is an indicator of both CYP1A1 and CYP1A2 [25,26].

Enzyme kinetic analysis indicated that the inhibition by piceatannol and *trans*-resveratrol was of mixed type (Table **1**). The apparent K_i values were similar for the inhibition of EROD activity by these compounds. In contrast, piceatannol inhibited MROD activity with a greater apparent K_i value, when compared to that by *trans*-resveratrol, suggesting that piceatannol was less potent than *trans*-resveratrol in the inhibition of hepatic microsomal MROD activity. By comparison, preincubation of microsomes with *trans*-resveratrol and NADPH did not affect EROD activity (data not shown), suggesting a lack of mechanism-based inactivation of rat CYP1A by the compound.

As shown in Table **2**, oral administration of *trans*-resveratrol (50 mg/kg) did not affect hepatic microsomal EROD or MROD activity at 10-60 min post-treatment in uninduced rats (i.e., no pretreatment) or 6-90 min in rats pretreated with BNF (as a means to enhance CYP1A-catalyzed biotransformation of *trans*-resveratrol to piceatannol) [10]. The levels of these enzyme activities were not significantly

Table 1. Enzyme Kinetic Analysis of Rat Hepatic Microsomal CYP1A Inhibition by Piceatannol and *trans*-Resveratrol

Enzyme Activity	Inhibitor	Apparent K_i (μM)	Mode of Inhibition
EROD	piceatannol	0.4 ± 0.03	mixed
	trans-resveratrol	0.4 ± 0.02	mixed
MROD	piceatannol	1.6 ± 0.14*	mixed
	trans-resveratrol	0.7 ± 0.06	mixed

The apparent K_i and mode of inhibition were determined by nonlinear regression analysis of the enzyme kinetic data as described under Materials and Methods. Results are expressed as mean ± SEM for four individual rats. *Significantly different from the *trans*-resveratrol group in the MROD assay, $p < 0.05$.

Table 2. Effect of Oral Administration of *trans*-Resveratrol on Rat Hepatic Microsomal CYP1A-Mediated EROD and MROD Activities

Time Post-Treatment (min)	EROD Activity (nmol/min/mg protein)	MROD Activity (nmol/min/mg protein)
Experiment 1 (no pretreatment)		
control	0.27 ± 0.01	0.12 ± 0.01
10	0.25 ± 0.01	0.11 ± 0.01
15	0.23 ± 0.02	0.10 ± 0.02
20	0.23 ± 0.02	0.11 ± 0.01
30	0.24 ± 0.02	0.10 ± 0.01
60	0.24 ± 0.02	0.10 ± 0.01
Experiment 2 (BNF pretreatment)		
control	11.0 ± 0.6	1.2 ± 0.1
6	10.8 ± 0.6	1.3 ± 0.1
12	9.8 ± 0.8	1.1 ± 0.1
18	11.0 ± 0.6	1.2 ± 0.1
24	9.6 ± 1.0	1.1 ± 0.1
Experiment 3 (BNF pretreatment)		
control	14.1 ± 1.3	1.7 ± 0.2
30	12.7 ± 1.0	1.5 ± 0.1
60	13.9 ± 0.3	1.6 ± 0.2
90	12.7 ± 1.4	1.6 ± 0.2

Adult male rats were not pretreated or pretreated i.p. with BNF at a dosage of 40 mg/kg once daily for three consecutive days. One day after the last dose of BNF, the rats were administered by oral gavage with *trans*-resveratrol (50 mg/kg) or the vehicle (20% hydroxypropyl γ-cyclodextrin / 2% Tween 80 in a ratio of 10:7, v/v). Rats were terminated at various times after the administration of *trans*-resveratrol. Hepatic microsomal EROD and MROD assays were performed as described in the Materials and Methods. Data are expressed as mean ± SEM enzyme activity for four individual rats per treatment group.

different between the rats treated with the vehicle or 0.9% NaCl (data not shown). A lack of an effect by *trans*-resveratrol was also obtained when the compound was administered i.p. (data not shown). Collectively, these findings are consistent with the *in vitro* results indicating that *trans*-resveratrol is not a mechanism-based inactivator of rat CYP1A. The analysis of hepatic microsomal enzyme activity has been used in previous studies to investigate the effect of *in vivo* administration of mechanism-based inactivators; for example, chloramphenicol [27], cimetidine [28], orphenadrine [29], and amiodarone [30].

trans-Resveratrol is metabolized extensively to glucuronide conjugates and, to a lesser extent, sulfate conjugates [20,31,32]. The systemic exposure to the *trans*-resveratrol aglycone is only 2.2% of the level of exposure to *trans*-resveratrol glucuronides [20], as determined by a comparison of the area under the plasma concentration-time curve. In rats administered orally with *trans*-resveratrol, the parent compound has been detected in hepatic tissues, but at levels (picomole quantities) [21] less than the *in vitro* apparent K_i values shown in the present study. Piceatannol has not been detected in plasma, urine, or tissues in rats administered *trans*-resveratrol. This reflects the extensive glucuronidation of *trans*-resveratrol [20] and the fact that piceatannol is also glucuronidated in rat liver [33]. Similarly, *trans*-resveratrol is biotransformed extensively and rapidly to glucuronide metabolites in human subjects [34]. Additional investigations will be required to determine whether the concentrations of *trans*-resveratrol achieved *in vivo* are sufficient to impair the clearance of CYP1 substrates and, if so, whether direct inhibition of CYP1 enzymes contributes to the chemoprotective action of *trans*-resveratrol reported in the previous rodent studies [11-13].

In summary, piceatannol decreased the *in vitro* catalytic activity of rat CYP1A1 and CYP1A2 by mixed inhibition. In contrast to the mechanism-based inactivation of human CYP1A2 by *trans*-resveratrol [14], this compound did not inactivate rat CYP1A enzymes. The reported species differences in mechanism-based inactivation of CYP enzymes highlight the need for detailed inhibition studies to be conducted with rodent and human enzymes at an early stage in drug development.

ACKNOWLEDGEMENTS

The authors thank Royalmount Pharma (Montreal, Quebec, Canada) for the generous provision of *trans*-resveratrol. This research was supported by Grant MOP-42385 (to T.K.H.C.) from the Canadian Institutes of Health Research. C.T.Y. was supported by a Kam Li Ma Traineeship in Pharmaceutical Sciences and a partial M.Sc. Traineeship in Drug Metabolism from Merck Research Laboratories (U.S.A.).

ABBREVIATIONS

BNF = β-naphthoflavone

CYP = Cytochrome P450

EROD = 7-ethoxyresorufin *O*-dealkylation

MROD = 7-methoxyresorufin *O*-dealkylation

Received: 08 August, 2006 Revised: 05 September, 2006 Accepted: 11 September, 2006

REFERENCES

[1] Kimura, Y.; Okuda, H.; Arichi, S. *Biochim. Biophys. Acta* **1985**, *834*, 275.

[2] Jayatilake, G. S.; Jayasuriya, H.; Lee, E. S.; Koonchanok, N. M.; Geahlen, R. L.; Ashendel, C. L.; McLaughlin, J. L.; Chang, C. J. *J. Nat. Prod.* **1993**, *56*, 1805.

[3] Langcake, P.; Pryce, R. J. *Physiol. Plant Pathol.* **1976**, *9*, 77.

[4] Ingham, I. L. *Phytochemistry* **1976**, *15*, 1791.

[5] Burns, J.; Yokota, T.; Ashihara, H.; Lean, M. E. J.; Crozier, A. *J. Agric. Food Chem.* **2002**, *50*, 3337.

[6] Aumont, V.; Krisa, S.; Battaglia, E.; Netter, P.; Richard, T.; Merillon, J. M.; Magdalou, J.; Sabolovic, N. *Arch. Biochem. Biophys.* **2001**, *393*, 281.

[7] Brill, S. S.; Furimsky, A. M.; Ho, M. N.; Furniss, M. J.; Li, Y.; Green, A. G.; Bradford, W. W.; Green, C. E.; Kapetanovic, I. M.; Iyer, L. V. *J. Pharm. Pharmacol.* **2006**, *58*, 469.

[8] Miksits, M.; Maier-Salamon, A.; Aust, S.; Thalhammer, T.; Reznicek, G.; Kunert, O.; Haslinger, E.; Szerkeres, T.; Jaeger, W. *Xenobiotica* **2005**, *35*, 1101.

[9] Potter, G. A.; Patterson, L. H.; Wanogho, E.; Perry, P. J.; Butler, P. C.; Ijaz, T.; Ruparelia, K. C.; Lamb, J. H.; Farmer, P. B.; Stanley, L. A.; Burke, M. D. *Br. J. Cancer* **2002**, *86*, 774.

[10] Piver, B.; Fer, M.; Vitrac, X.; Merillon, J. M.; Dreano, Y.; Berthou, F.; Lucas, D. *Biochem. Pharmacol.* **2004**, *68*, 773.

[11] Jang, M.; Cai, L.; Udeani, G. O.; Slowing, K. V.; Thomas, C. F.; Beecher, C. W. W.; Fong, H. H. S.; Farnsworth, N. R.; Kinghorn, A. D.; Mehta, R. G.; Moon, R. C.; Pezzuto, J. M. *Science* **1997**, *275*, 218.

[12] Banerjee, S.; Bueso-Ramos, C.; Aggarwal, B. B. *Cancer Res.* **2002**, *62*, 4945.

[13] Whitsett Jr., T. G.; Carpenter, D. M.; Lamartiniere, C. A. *J. Carcinog.* **2006**, *5*, 15.

[14] Chang, T. K. H.; Chen, J.; Lee, W. B. K. *J. Pharmacol. Exp. Ther.* **2001**, *299*, 874.

[15] Piver, B.; Berthou, F.; Dreano, Y.; Lucas, D. *Life Sci.* **2003**, *73*, 1199.

[16] Chun, Y. J.; Kim, M. Y.; Guengerich, F. P. *Biochem. Biophys. Res. Commun.* **1999**, *262*, 20.

[17] Schwarz, D.; Roots, I. *Biochem. Biophys. Res. Commun.* **2003**, *303*, 902.

[18] Yu, C.; Shin, Y. G.; Kosmeder, J. W.; Pezzuto, J. M.; van Breemen, R. B. *Rapid Commun. Mass Spectrom.* **2003**, *17*, 307.

[19] Chang, T. K. H.; Lee, W. B. K.; Ko, H. H. *Can. J. Physiol. Pharmacol.* **2000**, *78*, 874.

[20] Marier; J.F.; Vachon, P.; Gritsas, A.; Zhang, J.; Moreau, J. P.; Ducharme, M. P. *J. Pharmacol. Exp. Ther.* **2002**, *302*, 369.

[21] Asensi, M.; Medina, I.; Ortega, A.; Carretero, J.; Bano, M. C.; Obrador, E.; Estrela, J. M. *Free Radic. Biol. Med.* **2002**, *33*, 387.

[22] Lu, A. Y. H.; Levin, W. *Biochem. Biophys. Res. Commun.* **1972**, *46*, 1334.

[23] Chang, T. K. H.; Chen, J.; Benetton, S. A. *Drug Metab. Dispos.* **2002**, *30*, 378.

[24] Wolter, F.; Clausnitzer, A.; Akoglu, B.; Stein, J. *J. Nutr.* **2002**, *132*, 298.

[25] Nerurkar, P. V.; Park, S. S.; Thomas, P. E.; Nims, R. W.; Lubet, R. A. *Biochem. Pharmacol.* **1993**, *46*, 933.

[26] Burke, M. D.; Thompson, S.; Weaver, R. J.; Wolf, C. R.; Mayer, R. T. *Biochem. Pharmacol.* **1994**, *48*, 923.

[27] Halpert, J.; Balfour, C.; Miller, N. E.; Morgan, E. T.; Dunbar, D.; Kaminsky, L. S. *Mol. Pharmacol.* **1985**, *28*, 290.

[28] Chang, T.; Levine, M.; Bandiera, S. M.; Bellward, G. D. *J. Pharmacol. Exp. Ther.* **1992**, *260*, 1441.

[29] Reidy, G. F.; Mehta, I.; Murray, M. *Mol. Pharmacol.* **1989**, *35*, 736.

[30] Larrey, D.; Tinel, M.; Letteron, P.; Geneve, J.; Descatoire, V.; Pessayre, D. *Biochem. Pharmacol.* **1986**, *35*, 2213.

[31] Yu, C.; Shin, Y. G.; Chow, A.; Li, Y.; Kosmeder, J. W.; Lee, Y. S.; Hirschelman, W. H.; Pezzuto, J. M.; Mehta, R. G.; van Breemen, R. B. *Pharm. Res.* **2002**, *19*, 1907.

[32] Wenzel, E.; Somoza, V. *Mol. Nutr. Food Res.* **2005**, *49*, 472.

[33] Roupe, K.; Teng, X. W.; Fu, X.; Meadows, G. G.; Davies, N. M. *Biomed. Chromatogr.* **2004**, *18*, 486.

[34] Walle, T. *Free Radic. Biol. Med.* **2004**, *36*, 829.