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Effect of resveratrol on the pharmacokinetics of oral and intravenous nicardipine in rats: possible role of P-glycoprotein inhibition by resveratrol

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The present study aimed to assess the effect of resveratrol on the bioavailability of nicardipine in rats. Nicardipine was administered orally (12 mg kg^{-1}) or intravenously (4 mg kg^{-1}) with or without oral administration of resveratrol (0.5, 2.5 or 10 mg kg^{-1}). The oral administration of 2.5 or 10 mg kg^{-1} of resveratrol significantly increased both the area under the plasma concentration-time curve (AUC) ($P < 0.01$, 111–126%) and the peak plasma concentration (C_{max}) ($P < 0.01$, 105–121%), and significantly decreased the total body clearance (CL/F) ($P < 0.01$, 52.8–55.8%) of orally administered nicardipine. In contrast, resveratrol did not significantly change the pharmacokinetic parameters of i.v. nicardipine. Resveratrol significantly reduced rhodamine123 efflux via P-gp in MCF-7/ADR cells overexpressing P-gp. Resveratrol also inhibits CYP3A4, suggesting that the enhanced oral bioavailability of nicardipine by resveratrol may result from decreased P-gp-mediated efflux or inhibition of intestinal CYP3A4 metabolism. Based on these results, nicardipine dosage should be adjusted when given with supplements containing resveratrol.

1. Introduction

Nicardipine, a dihydropyridine calcium channel antagonist, causes coronary and peripheral vasodilatation by blocking the influx of extracellular calcium across cell membranes. Nicardipine is arterio-selective and effective for the treatment of hypertension, myocardial ischemia, and vasospasm in surgical patients (Kishi et al 1984; Hysing et al. 1986). Nicardipine has also been used experimentally to study the effects of calcium channel antagonists on the role of sympathetic nervous system activity in the development of cardiovascular risk (Van Swieten et al. 1997). The pharmacokinetics of nicardipine are non-linear due to hepatic first-pass metabolism, and show a bioavailability of about 35% following a 30 mg dose at steady state (Graham et al. 1984, 1985). Nicardipine is a substrate of P450 3A (CYP3A) subfamily enzymes, especially CYP3A4 in humans, and metabolized to pharmacologically inactive forms (Higuchi and Shiobara 1980; Guengerich 1991; Guengerich et al. 1986). In addition, nicardipine is a P-glycoprotein (P-gp) substrate (Wang et al. 2000; Hu et al. 1996).

Resveratrol (*trans*-3′,4′,5-trihydroxy-stilbene), a phytoalexin present in mulberries, peanuts, and grapes, is regularly consumed in the human diet (Soleas et al. 1997a). It is synthesized by grapes in response to fungal infections and is found, therefore, in red wine at levels between 1 and 10 μM (Soleas et al. 1997b). Resveratrol may be responsible for the beneficial effects of moderate wine consumption in the prevention of cardiovascular diseases (Constant 1997) via its antioxidant and anticoagulant properties (Fauconneau et al. 1997). Resveratrol modulates the synthesis of hepatic apolipoprotein and lipids and inhibits platelet aggregation and eicosanoid production in human platelets and neutrophils (Bertelli et al. 1996). Moreover, resveratrol inhibits events associated with tumor initiation, promotion, and progression (Jang et al. 1997). Resveratrol irreversibly inhibits CYP3A4 with a Ki of 20 μM (Chan and Delucchi 2000; Piver et al. 2001). Moreover, resveratrol increases the accumulation of daunorubicin (a P-gp substrate) in KB-C2 cells in a concentration-dependent manner, indicating that resveratrol also inhibits P-gp (Nabekura et al. 2005).

The low bioavailability of oral nicardipine is mainly due to pre-systemic metabolism and P-gp mediated efflux in the intestine. Resveratrol, a dual inhibitor of CYP 3A4 and P-gp, might improve the pharmacokinetics of nicardipine in combination therapy, although adverse effects may occur if doses are not appropriate. Therefore, we determined whether resveratrol administration affects the pharmacokinetics of nicardipine in rats and also assessed whether resveratrol inhibit P-gp activity in P-gp overexpressing MCF-7/ADR cells.

2. Investigations, results and discussion

The plasma concentration-time profiles of nicardipine after oral administration at a dose of 12 mg kg^{-1} in rats pretreated with or without resveratrol (0.5, 2.5 or 10 mg kg^{-1}) are shown in Fig. 1, and the pharmacokinetic parameters of oral nicardipine are summarized in Table 1. Resveratrol (2.5 or 10 mg kg^{-1}) significantly ($P < 0.01$) increased the area under the plasma concentration-time curve (AUC) of nicar-

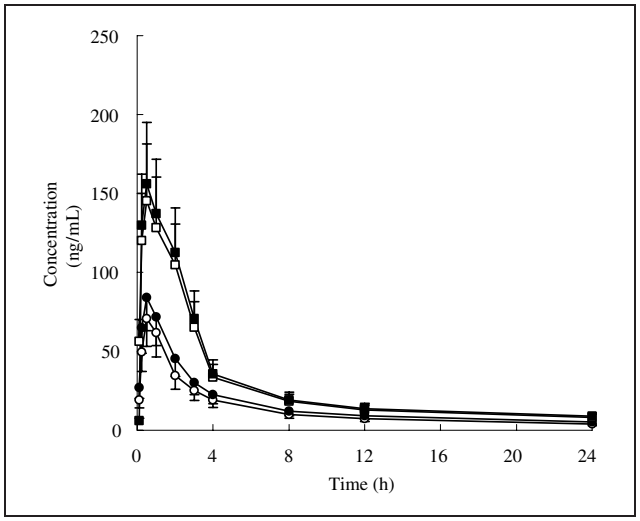

Fig. 1: Mean plasma concentration-time profiles of nicardipine after oral administration of nicardipine (12 mg kg^{-1}) to rats with or without resveratrol (0.5, 2.5 or 10 mg kg^{-1}). Bars represent the standard deviation (n = 6), (○) 12 mg kg^{-1} of oral nicardipine, (●) with 0.5 mg kg^{-1} of resveratrol, (□) with 2.5 mg kg^{-1} of resveratrol, (■) with 10 mg kg^{-1} of resveratrol

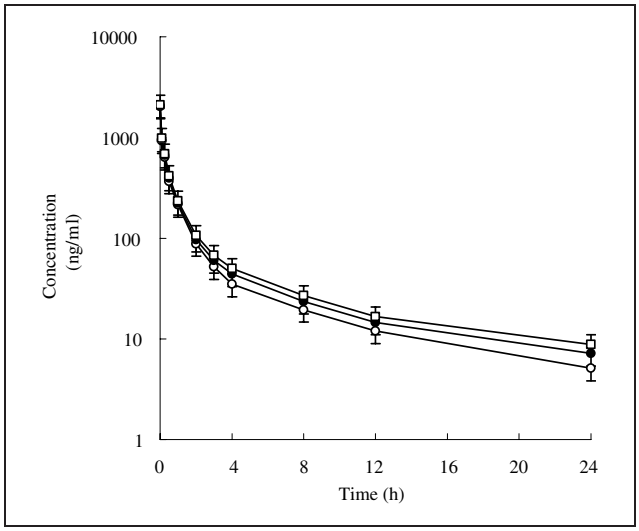

Fig. 3: Mean plasma concentration–time profiles of nicardipine after i.v. administration of nicardipine (4 mg kg^{-1}) to rats with or without resveratrol (2.5 or 10 mg kg^{-1}). Bars represent the standard deviation (n = 6), (○) 4 mg kg^{-1} of i.v. nicardipine, (□) with 2.5 mg kg^{-1} of resveratrol, (■) with 10 mg kg^{-1} of resveratrol

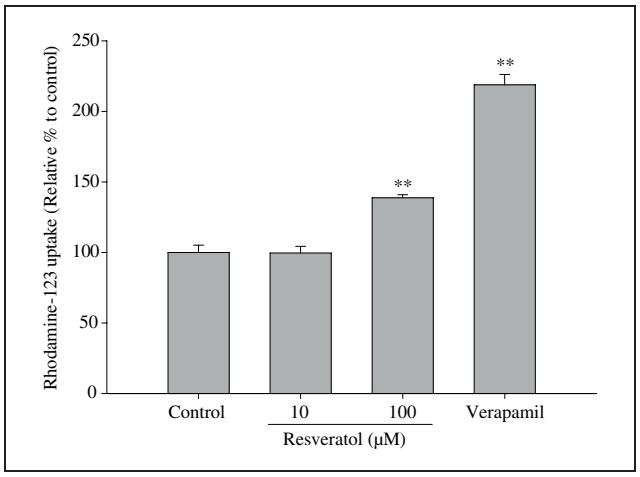

Fig. 2: Rhodamine-123 retention. After incubation of MCF-7/ADR cells with 20 μM R-123 for 90 min, the R-123 fluorescence values in cell lysates were measured using the excitation and emission wavelengths of 480 and 540 nm, respectively. The values were divided by total protein contents of each sample. Bars represent the standard deviation (n = 4), (significant versus the control MCF-7 cells, $^{**}p < 0.01$). Verapamil, a P-gp inhibitor (100 μM) was used as a positive control

dipine by 111% to 126%, respectively, and the peak concentration (C_{max}) of nicardipine was also enhanced by 105% to 121%, whereas the total plasma clearance (CL/F) of nicardipine was significantly ($P < 0.01$) decreased by resveratrol (2.5 or 10 mg kg^{-1}). Consequently, the relative bioavailability of nicardipine was increased 2.12 or 2.26 fold by 2.5 or 10 mg kg^{-1} resveratrol. Resveratrol did not change the K_{el}, T_{max}, or $t_{1/2}$ of orally administered nicardipine.

CYP3A4 is the most predominant P450 present in the small intestine (Kolars et al. 1992) and resveratrol inactivates CYP3A4 in the presence of NADPH (Chan et al. 2000; Chang et al. 2001). Hence, the enhanced oral bioavailability of nicardipine after resveratrol treatment might be due to the inactivation of CYP3A4 in the liver and/or intestine. Oral nicardipine can also be affected by P-gp-mediated efflux in the small intestine. Resveratrol (100 μM) increased rhodamine retention in P-gp over-expressing MCF-7/ADR cells by 140% (Fig. 2), indicating inhibition of P-gp activity. This is partly consistent with a recent publication (Nabekura et al. 2005). Thus, the increase in oral nicardipine bioavailability induces by resveratrol could indicate decreased P-gp efflux in the intestine. However, a lower dose (0.5 mg kg^{-1}) of resveratrol did

Table 1: Mean (± S.D.) pharmacokinetic parameters of nicardipine after oral administration of nicardipine (12 mg kg^{-1}) to rat with or without resveratrol

Parameters	Control	Nicardipine + resveratrol		
		0.5 mg kg^{-1}	2.5 mg kg^{-1}	10 mg kg^{-1}
AUC (ng mL^{-1} h)	370 ± 91.2	463 ± 142	783 ± 169**	838 ± 205**
C_{max} (ng mL^{-1})	70.7 ± 17.5	84.1 ± 24.5	145 ± 35.8**	156 ± 38.1**
T_{max} (h)	0.5	0.5	0.5	0.5
CL/F (mL min^{-1} kg^{-1})	541 ± 135	432 ± 131	256 ± 63.3**	239 ± 58.3**
K_{el} (h^{-1})	0.072 ± 0.018	0.067 ± 0.016	0.065 ± 0.015	0.064 ± 0.014
$t_{1/2}$ (h)	9.6 ± 2.40	10.3 ± 2.56	10.7 ± 2.57	10.8 ± 2.72
R.B. (%)	100	125	212	226

Mean ± S.D., n = 6. ** $P < 0.01$, compared with the control group
AUC: area under the plasma concentration-time curve from 0 h to infinity, C_{max}: peak plasma concentration, T_{max}: time to reach peak concentration, CL/F: total plasma clearance, K_{el}: elimination rate constant, $t_{1/2}$: terminal half-life, R.B.: the relative bioavailability

Table 2: Mean (± S.D.) pharmacokinetic parameters of nicardipine after intravenous administration of nicardipine (4 mg kg^{-1}) to rats with or without resveratrol

Parameters	Control	Nicardipine + resveratrol	
		2.5 mg kg^{-1}	10 mg kg^{-1}
AUC (ng mL^{-1} h)	1133 ± 312	1280 ± 319	1399 ± 351
CL_t (mL min^{-1} kg^{-1})	58.9 ± 15.1	52.1 ± 12.9	47.7 ± 11.3
K_{el} (h^{-1})	0.093 ± 0.025	0.086 ± 0.020	0.082 ± 0.019
$t_{1/2}$ (h)	7.5 ± 1.86	8.1 ± 2.03	8.4 ± 2.14

Mean ± S.D., n = 6
AUC: area under the plasma concentration-time curve from 0 h to infinity; CL_t: total plasma clearance; K_{el}: elimination rate constant; $t_{1/2}$: terminal half-life

not significantly change the pharmacokinetic parameters of nicardipine, which implies that a higher dose of resveratrol is required for the inhibition of P-gp or CYP3A4.

Figure 3 shows the plasma concentration-time profiles of nicardipine after i.v. injection (4 mg kg^{-1}) in rats with or without resveratrol (2.5 or 10 mg kg^{-1}). Resveratrol (2.5 or 10 mg kg^{-1}) did not significantly change the pharmacokinetic parameters of i.v. nicardipine (Table 2). Hence, the intestine is the primary site of resveratrol inhibition of CYPs or P-gp.

In conclusion, the oral administration of resveratrol (2.5 or 10 mg kg^{-1}) enhanced the bioavailability of nicardipine. Therefore, concomitant use of resveratrol supplements with nicardipine requires close monitoring for potential drug interactions.

3. Experimental

3.1. Chemicals and apparatus

Nicardipine, resveratrol and nimodipine (an internal standard for HPLC analysis for nicardipine) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). HPLC grade acetonitrile was acquired from Merck Co. (Darmstadt, Germany). Other chemicals for this study were of reagent grade.

The apparatus used in this study was an HPLC equipped with a Waters 1515 isocratic HPLC Pump, a Waters 717 plus autosampler with a Waters™ 474 scanning fluorescence detector (Waters Co., Milford, MA, USA), a HPLC column temperature controller (Phenomenex Inc., CA, USA), a Bransonic® Ultrasonic Cleaner (Branson Ultrasonic Co., Danbury, CT, USA), a vortex-mixer (Scientific Industries Co., NY, USA) and a high-speed micro centrifuge (Hitachi Co., Tokyo, Japan).

3.2. Animal experiments

Male Sprague-Dawley rats at 7–8 weeks of age (weighing 270–300 g) were purchased from Dae Han Laboratory Animal Research Co. (Choongbuk, Republic of Korea) and given free access to a commercial rat chow diet (No. 322-7-1; Superfeed Co., Gangwon, Republic of Korea) and tap water. The animals were housed (two rats per cage) in a clean room maintained at a temperature of 22 ± 2 °C and relative humidity of 50–60%, with 12 h light and dark cycles. The rats were acclimated under these conditions for at least 1 week. All animal studies were performed in accordance with the "Guiding Principles in the Use of Animals in Toxicology" adopted by the Society of Toxicology (USA), and the Animal Care Committee of Chosun University (Gwangju, Republic of Korea) approved the protocol of this animal study. The rats were fasted for at least 24 h prior to beginning the experiments and had free access to tap water. Each animal was anaesthetized lightly with ether. The left femoral artery and vein were cannulated using polyethylene tubing (SP45, I.D. 0.58 mm, O.D. 0.96 mm; Natsume Seisakusho Co. LTD., Tokyo, Japan) for blood sampling and i.v. injection, respectively.

3.3. Oral and intravenous administration of nicardipine

The rats were divided into six groups (n = 6, each); an oral group (12 mg kg^{-1} of nicardipine dissolved in water; homogenized at 36 °C for 30 min; 3.0 mL kg^{-1}) without (control) or with 0.5, 2.5 or 10 mg kg^{-1} of oral resveratrol, and an i.v. group (4 mg kg^{-1} of nicardipine, dissolved in 0.9% NaCl solution; homogenized at 36 °C for 30 min; 1.5 mL kg^{-1}) without (control) or with 2.5 or 10 mg kg^{-1} of oral resveratrol. Oral nicardipine was administered through a feeding tube, and resveratrol was intragastrically administered 30 min prior to oral or intravenous administration of nicardipine. Nicardipine for i.v. administration was injected through the femoral vein within 0.5 min. A 0.45 mL blood sample was collected into heparinized tubes from the femoral artery at 0 (to serve as a control), 0.017 (at the end of infusion), 0.1, 0.25, 0.5, 1, 2, 3, 4, 8, 12 and 24 h after intravenous infusion, and 0.1, 0.25, 0.5, 1, 2, 3, 6, 8, 12 and 24 h for oral study. The blood samples were centrifuged at 13,000 rpm for 5 min, and the plasma samples were stored at −40 °C until HPLC analysis of nicardipine. Approximately 1 mL of whole blood collected from untreated rats was infused via the femoral artery at 0.25, 1, 3 and 8 h to replace the blood loss due to blood sampling.

3.4. HPLC assay

The plasma concentrations of nicardipine were determined by a HPLC assay method reported by Eastwood et al. (1990). Briefly, 50 μL of nimodipine (2 μg mL^{-1}), 20 μL of 2 N sodium hydroxide solution, and 1.2 mL of tert-butylmethylether : Hexane (75 : 25) were added to a 0.2 mL of the plasma sample. The mixture was then stirred for 2 min and centrifuged at 13,000 rpm for 10 min. The organic layer (1 mL) was transferred to a clean test tube and evaporated at 35 °C under a stream of nitrogen. The residue was dissolved in 200 μL of the mobile phase and centrifuged (13,000 rpm, 5 min). The supernatant (50 μL) was injected into the HPLC system. Chromatographic separation was achieved using a Symmetry® C_{18} column (4.6 × 150 mm, 5 μm, Waters Co.), and a μBondapak™ C_{18} HPLC Precolumn (10 μm, Waters Co.). The mobile phase was acetonitrile: 0.015 M KH_2PO_4 (60 : 40, v/v, PH 4.5) with 2.8 mM triethylamine, which was run at a flow rate of 1.5 mL min^{-1}. Chromatography was performed at a temperature of 30 °C that was set by an HPLC column temperature controller. The UV detector was set to 254 nm. The retention times of nicardipine and the internal standard were 7.8 and 4.2 min, respectively (Fig. 4). The detection limits of nicardipine in rat plasma was 10 ng mL^{-1}. The coefficients of variation for nicardipine were below 14.1%.

3.5. Pharmacokinetic analysis

The plasma concentration data were analyzed with a non-compartmental method using WinNonlin software version 4.1 (Pharsight Co., Mountain View, CA, USA). The elimination rate constant (K_{el}) was calculated by log-linear regression of nicardipine concentration data during the elimina-

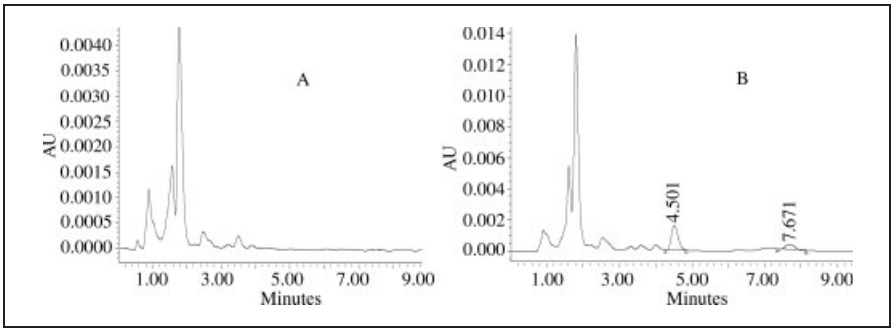

Fig. 4: Chromatograms of blank plasma (A) and plasma spiked (B) with internal standard (IS, 4.5 min) and nicardipine (7.7 min)

tion phase, and the terminal half-life ($t_{1/2}$) was calculated by $0.693/K_{el}$. The peak concentration (C_{max}) and the time to reach peak concentration (T_{max}) of nicardipine in plasma were obtained by visual inspection of the data from the concentration-time curve. The area under the plasma concentration-time curve (AUC_{0-t}) from time zero to the time of last measured concentration (C_{last}) was calculated by the linear trapezoidal rule. The AUC zero to infinite ($AUC_{0-\infty}$) was obtained by the addition of AUC_{0-t} and the extrapolated area determined by C_{last}/K_{el}. Total body clearance (CL/F) was calculated by Dose/AUC. The relative bioavailability of nicardidipine was estimated by $AUC_{combined}/AUC_{control}$.

3.6. Rhodamine-123 retention assay

MCF-7/ADR cells were seeded in 24-well plates. At 80% confluence, the cells were incubated in FBS-free DMEM for 18 h. The culture medium was changed to Hanks' balanced salt solution and the cells were incubated at 37 °C for 30 min. After incubation of the cells with 20 μM rhodamine-123 in the presence or absence of nicardipine (100 μM) and resveratrol (10 or 100 μM) for 90 min, the medium was completely removed. The cells were then washed three times with an ice-cold phosphate buffer (pH 7.0) and lysed in EBC lysis buffer. The rhodamine-123 fluorescence in the cell lysates was measured using excitation and emission wavelengths of 480 and 540 nm, respectively. Fluorescence values were normalized to the total protein content of each sample and presented as the ratio to controls.

3.7. Statistical analysis

All mean values are presented with standard deviation (Mean ± S.D.). Statistical analysis was conducted using one-way ANOVA followed by *a posteriori* testing with Dunnett's correction. Differences were considered significant at a level of $P < 0.05$.

References

Bertelli AAE, Giovanni L, Stradi R, Urien S, Tillement JP, Bertelli A (1996) Kinetics of trans- and cis-resveratrol (3,4′,5-trihydroxystilbene) after red wine oral administration in rats. Int J Clin Pharmacol Res 26: 77–81.

Chan WK, Delucchi AB (2000) Resveratrol, a red wine constituent, is a mechanism-based inactivator of cytochrome P450 3A4. Life Sci 67: 3103–3112.

Chang TK, Yeung RK (2001) Effect of trans-resveratrol on 7-benzyloxy-4-trifluoromethylcoumarin O-dealkylation catalyzed by human recombinant CYP3A4 and CYP3A5. Can J Physiol Pharmacol 79: 220–226.

Constant J (1997) Alcohol, ischemic heart disease, and the French paradox. Coronary Artery Dis 8: 645–649.

Eastwood RJ, Galustian C, Bhamra RK, Holt DW (1990) High-performance liquid chromatographic method for the measurement of nicardipine in plasma or serum. J Chromatogr 530: 463–468.

Fauconneau B, Waffo-Teguo P, Huguet F, Barrier L, Decendit A, Merillon JM (1997) Comparative study of radical scavenger and antioxidant properties of phenolic compounds from *Vitis vinifera* cell cultures using *in vitro* tests. Life Sci 61: 2103–2110.

Graham DJ, Dow RJ, Freedman D, Mroszczak E, Ling T (1984) Pharmacokinetics of nicardipine following oral and intravenous administration in man. Postgrad Med J 4: 7–10.

Graham DJ, Dow RJ, Hall DJ, Alexander OF, Mroszczak EJ, Freedman D (1985) The metabolism and pharmacokinetics of nicardipine hydrochloride in man. Br J Clin Pharmacol 1: 23–28.

Guengerich FP (1988) Roles of cytochrome P-450 enzymes in chemical carcinogenesis and cancer chemotherapy. Cancer Res 48: 2946–2954.

Guengerich FP, Martin MV, Beaune PH, Kremers P, Wolff T, Waxman DJ (1986) Characterization of rat and human liver microsomal cytochrome P-450 forms involved in nifedipine oxidation, a prototype for genetic polymorphism in oxidative drug metabolism. J Biol Chem 261: 5051–5060.

Higuchi S, Shiobara Y (1980) Metabolic fate of nicardipine hydrochloride, a new vasodilator, by various species *in vitro*. Xenobiotica 10: 889–896.

Hu YP, Chapey C, Robert J (1996) Relationship between the inhibition of azidopine binding to P-glycoprotein by MDR modulators and their efficiency in restoring doxorubicin intracellular accumulation. Cancer Lett 109: 203–209.

Hysing ES, Chelly JE, Doursout MF, Hartley C, Merin RG (1986) Cardiovascular effects of and interaction between calcium blocking drugs and anesthetics in chronically instrumented dogs. III. Nicardipine and isoflurane. Anaesthesiology 65: 385–391.

Jang M, Cai L, Udeani GO, Slowing KV, Thomas CF, Beecher CWW et al. (1997) Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. Science 275: 218–220.

Kishi Y, Okumura F, Furuya H (1984) Haemodynamic effects of nicardipine hydrochloride. Studies during its use to control acute hypertension in anaesthetized patients. Br J Anaesth 56:1003–1007.

Kolars JC, Schmiedlin-Ren P, Schuetz JD, Fang C, Watkins PB (1992) Identification of rifampin-inducible P450IIIA4 (CYP3A4) in human small bowel enterocytes. J Clin Invest 90: 1871–1878.

Nabekura T, Kamiyama S, Kitagawa S (2005) Effects of dietary chemopreventive phytochemicals on P-glycoprotein function. Biochem Biophys Res Commun 327: 866–870.

Piver B, Berthou F, Dreano Y, Lucas D (2001) Inhibition of CYP3A, CYP1A and CYP2E1 activities by resveratrol and other non volatile red wine components. Toxicol Lett 125: 83–91.

Soleas G.J, Diamandis EP, Golberg DM (1997b) Wine as a biological fluid: history, production, and role in disease prevention. J Clin Lab Anal 11: 287–313.

Soleas GJ, Diamandis EP, Golberg DM (1997a) Resveratrol: a molecule whose time has come? and gone? Clin Biochem 30: 91–113.

Van Swieten PA, Hansson L, Epstein M (1997) Blood Press 6: 78–90.

Wang EJ, Casciano CN, Clement RP, Johnson WW (2000) Two transport binding sites of P-glycoprotein are unequal yet contingent: initial rate kinetic analysis by ATP hydrolysis demonstrates intersite dependence. Biochim Biophys Acta 1481: 63–74.