Heterogeneity and Standardization of Phase II Metabolism in Cultured Cells

Lucia Biasutto^{1,2}, Ester Marotta², Umberto De Marchi¹, Silvia Beltramello¹, Alice Bradaschia¹, Spiridione Garbisa¹, Mario Zoratti^{1,3} and Cristina Paradisi²

¹Department of Biomedical Sciences, University of Padova, ²Department of Chemical Sciences, University of Padova, ³CNR Institute of Neuroscience, Padova

Key Words

Caco-2 • Phase II metabolism • Sulfotransferase • Glucuronosyltransferase • Methyltransferase • SULT • UGT • COMT • Detoxification • Xenobiotics

Abstract

Caco-2 cells are widely used for transepithelial transport and metabolism studies. We analysed the metabolites produced from quercetin (Q) during transport of this flavonoid across Caco-2 monolayers and by plastic-adhering cells. We found that the pattern of Phase II metabolic activity varies markedly depending on the particular cell clone, age of the cell culture, and stressful treatment such as freezing/thawing. Prolonged culturing and stress cause a decrease of “detoxifying” conjugating activity. This can be re-established by growing the cells with a low concentration of the transport/metabolism substrate for a few days. We suggest this metabolism-activating procedure be used to make studies with these cells more readily comparable.

Introduction

Caco-2 cells [1-4] are very popular as a convenient stand-in for intestinal epithelium in transport studies. They are a human epithelial colon cancer cell line originating from a colon adenocarcinoma having both enterocytic and colonocytic characteristics. When cultured as a monolayer, they differentiate forming tight junctions and mimicking the villous intestinal epithelium in several respects. If formed on a porous support, such as a Transwell® filter, the monolayer separates an apical from a basolateral compartment, both accessible to the experimenter, and allows to study the kinetics, mechanism and pharmacology of the passage of any given substance from one side to the other. They are most often reductively considered as a permeability barrier, but in addition to adhesion molecules, transporters and efflux “pumps” they can express Phase II metabolism enzymes: UDP-glucuronosyltransferases UGT1 and UGT2 [5, 6], sulfotransferases SULT1 and SULT2 [7-9] and methyl transferases [10, 11]. The expression of these enzymes makes Caco-2 cells potentially useful to study biotransformations taking place during the intestinal permeation step of the absorption of a drug or nutrient. Although the liver plays an even greater role in the “detoxification” of xenobiotics, the metabolic

Copyright © 2009 S. Karger AG, Basel

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

© 2009 S. Karger AG, Basel
1015-8987/09/0236-0425\$26.00/0

Accessible online at:
www.karger.com/cpb

Dr. Mario Zoratti
CNR Institute of Neuroscience
c/o Dept. of Biomedical Sciences
Viale G. Colombo 3, 35121 Padova (Italy)
Tel: +39 0498276054, Fax +39 0498276049, E-Mail zoratti@bio.unipd.it

role of the intestine is being increasingly recognized as crucial (e.g. [12]), especially since metabolic transformations are linked to bioavailability via the interplay with transport by MDR-family efflux proteins (e.g. [13-15]). Polyphenols, with their multiple OH groups, are ready-made substrates for Phase II enzymes, and the resulting metabolites are known to be exported from enterocytes via transporters of the MDR family (e.g. [15-22]).

Several studies have established a correlation between Caco-2 monolayer permeability and performance in other models of intestinal absorption (e.g. [23] and refs. therein). Variations in results from laboratory to laboratory and from cell batch to cell batch have however led to the realization that "Caco-2" has actually come to denote a collection of subpopulations yielding monolayers with different transport properties, depending also on such factors as cell batch, age (number of cell cycles), growth conditions and so forth. This heterogeneity seems to reflect in part that of the originally established culture. A few Caco-2 clones with greater homogeneity have been isolated and characterized [24]. This variability in transport/permeability characteristics has been described in detail by several authors and in a couple of excellent recent reviews [23, 25].

Much less attention has been paid to the variability in metabolic ability. This aspect should however be of just as much concern, since permeation and metabolism are related in absorbing epithelia. We report here our observations concerning this variability, and a simple procedure to re-establish latent metabolic ability in deficient cell populations. The latter was based on the notion that expression of detoxifying enzymes may be induced by prolonged exposure to polyphenols. For example in the case of resveratrol and HepG2 cells exposure to 10 μ M resveratrol for 24 hours induced a considerable increase in mRNA and protein expression for two isoforms of UDP-glucuronosyltransferases and a sulfotransferase [26]. A similar potentiation of Phase II detoxifying enzymes was observed in the liver of rats fed resveratrol [27].

Materials and Methods

Materials

All chemicals for buffer preparations were of laboratory grade, obtained from J. T. Baker, Merck, or Sigma. The composition of HBSS was as follows (in mM units): NaCl 136.9, KCl 5.36, CaCl₂ 1.26, MgSO₄ 0.81, KH₂PO₄ 0.44, Na₂HPO₄ 0.34, Glucose 5.55, pH 7.4 (with NaOH). Corning-Costar Transwell® Clear plates (12- or 6-well, polyester membrane, 12 or 24 mm

insert diameter, 1.0 or 4.7 cm² septum surface, respectively, 0.4 μ m pore size) were purchased from Cellbio (Milan). Caco-2 cell lines were kindly provided by the groups of M. De Bernard, S. Garbisa and E. Papini of the Dept. of Biomedical Sciences, University of Padova. Another Caco-2 clone, originally from ATCC, was purchased from the Istituto Zooprofilattico di Brescia (Italy).

Cell culture

Cells were seeded in culture flasks and passaged in Dulbecco's Modified Eagle's medium (DMEM) (GIBCO) supplemented with 10% fetal bovine serum (Biospa), 1% penicillin/streptomycin solution (10000 U/mL and 10 mg/mL, respectively, in PBS), 1% glutamine (200 mM in PBS), 1% nonessential amino acids (100 $\times$ solution) and 1% Hepes (1 M in PBS). For freezing, cells grown to near confluence in a 75 cm² culture flask were centrifuged and resuspended in 750 μ L fetal bovine serum. 250 μ L of a DMEM:DMSO, 3:2 mixture were then added very slowly; the cell suspension was transferred into a vial, allowed to freeze at -80°C and then transferred into liquid N₂ for storage.

Monolayer formation

Procedures were as described previously [28]. The cells were seeded onto tissue culture treated Transwell Clear® inserts at a density of 3×10^5 cells $\times$ cm⁻². Monolayers were grown in a humidified atmosphere with 5% CO₂ at 37°C. Trans Epithelial Resistance (TER) was measured periodically using a Millipore Millicell-ERS epithelial voltohmmeter and experiments were performed when values reached approximately 1500-2000 $\Omega \times$ cm² (10-20 days post-seeding).

Transport across cell monolayers

The initial stock solution of quercetin was freshly made in DMSO and was then diluted with HBSS to give formally 50 μ M solutions (loading solution; final DMSO concentration 0.1%), and sonicated to obtain a finer dispersion of undissolved suspended material. The cells monolayers supported on Transwell® septa were washed twice with warm HBSS. The loading solution was added to the apical side (0.5 or 1.5 mL for 12-mm and 24-mm septa, respectively), while HBSS was added on the basolateral side (1.5 and 2.6 mL, respectively). At the desired time, apical and basolateral solutions were transferred into glass vials and frozen for analysis by HPLC-UV and LC-MS. Basolateral solutions were analyzed without any treatment; apical solutions were diluted with an equal volume of CH₃CN and filtered through 0.45 μ m PTFE filters (Chemtek Analytica) to eliminate cell residues. HPLC-UV and LC-MS analyses were carried out as described below.

Metabolism studies

Cells growing in flasks were seeded onto 6-well plates at a density of 6×10^5 cells per well, and were grown until they reached 100% confluence (5 days). To evaluate metabolic activity, they were then washed with warm HBSS, and incubated for 8 h with 1 mL/well of a 20 μ M freshly prepared solution of quercetin (dilution of a 1000 $\times$ stock solution in DMSO; 0.1% final DMSO) in DMEM without phenol red (to avoid interfer-

Fig. 1. A comparison of the conjugating activity of four Caco-2 clones. Relative abundance of quercetin and its major Phase II metabolites (sulfate- and methyl- quercetin) found after 6 hours in the basolateral compartment of Transwell®-supported monolayers exposed to (formally) 50 μ M quercetin on the apical side (representative experiment). The sum of the concentrations of all compounds is considered as 100%. Error bars indicate standard deviation (N = 4 for clone A; N = 3 for clones C and D; N = 1 for clone B).

ence in HPLC analyses). Medium and cells were collected together after 8 hours of incubation. 100 μ L of 0.6 M acetic acid and 100 μ L of 10 mM ascorbic acid (freshly prepared solution) were added, and the samples were immediately stored at -20°C until treatment and analysis. Treatment consisted in addition of acetone (1 mL), followed by sonication (2 min), filtration through 0.45 μ m PTFE syringe filters and concentration under N_2 .

Induction of conjugating enzymes

Cells were grown for 3 or 6 days in culture medium supplemented with 1 μ M quercetin (dilution from a 1000 $\times$ stock solution, 0.1% final DMSO). The medium was replaced with fresh one every day. 5 days before the planned experiment the cells were seeded onto 6-well plates as above. Metabolism experiments were performed at the end of the 5-day period (confluence), which coincided with the end of the 3- or 6-day quercetin treatment.

HPLC-UV and LC-ESI/MS

HPLC-UV analyses were performed by a Thermo Separation Products Inc. system with a P2000 Spectra System pump and a UV6000LP diode array detector (190–500 nm). The sample solution (20 μ L) was injected into a reversed phase column (Synergi-Max, 4 μ m, 150 $\times$ 4.6 mm i.d.; Phenomenex). Solvent A was $\text{H}_2\text{O}:\text{CF}_3\text{COOH}$ (99.9:0.1, v:v) and B was CH_3CN . The flow rate was 1 mL/min, the gradient for B was as follows: 17% (2 min), 25% (in 5 min), 35% (in 8 min), 50% (in 5 min), 100% (in 15 min). The eluate was preferentially monitored at 270, 300 and 370 nm. Due to their spectral characteristics, quercetin and its derivatives were best observed at 370 nm. Quercetin metabolites have UV-Vis spectra very similar to that of quercetin (Biasutto,

Fig. 2. Metabolic characteristics do not depend on cell density at seeding or on the value of Trans Epithelial Resistance (TER) of the differentiated monolayer. Shown is the relative abundance of quercetin and its major Phase II metabolites (sulfate- and methyl- quercetin) found after 6 hours on the basolateral side of Transwell®-supported monolayers exposed to (formally) 50 μ M quercetin on the apical side (representative experiments). Shown are representative experiments exemplifying a general observation (N = 16). A) Data from two experiments with the same clone (clone D). Cells were seeded onto Transwell® inserts at two densities (1.5×10^5 and 3×10^5 cells per cm^2), producing monolayers with TER values of 1800 and 3000 $\Omega \times \text{cm}^2$ at the time of the transport activity evaluation (13 days from seeding). B) The data shown are from: clone A: three experiments with average TER value of $1924 \pm 203 \Omega \times \text{cm}^2$; clone C: one experiment with TER = 1800 $\Omega \times \text{cm}^2$; clone D: three experiments with average TER value of $2150 \pm 223 \Omega \times \text{cm}^2$.

L. et al., unpublished), and the same value of $\epsilon_{370 \text{ nm}}$ was used for quantification.

LC-MS was performed on selected samples with a 1100 Series Agilent Technologies system, equipped with binary pump (G1312A), diode array detector (G1315B) and MSD SL Trap mass spectrometer (G2445D SL) with ESI source operating in full-scan mode in both positive and negative ion mode. The column, the solvents and the gradient profile were the same as used for HPLC-UV analyses. Before LC-MS analysis, samples were further concentrated under vacuum (about 10 $\times$). Metabolites were identified by their UV-Vis and mass spectra. For the purposes of this paper sulfate and methylsulfate derivatives are considered together as “sulfate”.

Fig. 3. Loss of metabolic activity. Relative abundance of quercetin and its major Phase II metabolites found after 6 hours on the basolateral side of Transwell®-supported monolayers exposed to (formally) 50 μ M quercetin on the apical side. Experiments with clone A at different times: a) after only a few cell divisions after receipt of the cells; b) after culture for about 6 months; c) cells as in a) but after freezing and storage in liquid N_2 . A) HPLC chromatograms ($\lambda = 370$ nm) of samples from representative experiments. B) Column plot of the averages obtained from 4, 3 and 5 experiments for a), b) and c) respectively.

Results

The observations reported here were collected using four Caco-2 cell lines obtained from different sources. When used as received in monolayer transport experiments, one of these lines ("clone A") exhibited strong phase II conjugation activity, resulting in the presence in the basolateral compartment mostly of quercetin sulfate. Three other lines (B-D) had only weak methyl- and sulfotransferase activities, with quercetin being the main compound both in the apical and basolateral side. The "clone A" line tended to lose conjugating activity as the number of passages increased, and needed to be periodically re-established from frozen stocks. However in several instances we observed a loss of conjugative activity apparently induced by freezing and storage of the cells in liquid N_2 . Fig. 1 illustrates this variability by plotting the relative abundance of the major Phase II metabolites

Fig. 4. Recovery of sulfotransferase activity. A) HPLC Chromatograms (370 nm) and B) relative abundance of quercetin and its major Phase II metabolites found in the medium and cells exposed to 20 μ M Q for 8 hours. The data come from a representative experiment (out of 5) obtained with the same clone cultured for different time periods, as indicated, in the presence of 1 μ M quercetin in the culture medium (see Materials and Methods). QMG: quercetin- methyl glucuronide; QMS: quercetin- methyl sulfate; QS: quercetin- sulfate; Q: quercetin; QM: methyl- quercetin. The compounds were identified by LC-MS.

found after 6 hours on the basolateral side of Transwell®-supported monolayers exposed to (formally) 50 μ M quercetin on the apical side (representative experiments with the 4 clones).

The different metabolic behaviour did not depend on the number of seeded cells (Fig. 2A) or tightness of the differentiated monolayer (Fig. 2A, B). Data obtained with clone A in different time periods suggested a possible loss in expression of conjugating enzymes; the cells initially had considerable conjugating activity, but lost it after a undergoing many divisions or, in some cases, after freezing (Fig. 3).

It would be desirable to perform experiments with cells endowed with comparable metabolic activities. We therefore checked whether metabolism could be re-established by culture in the presence of low concentrations of the xenobiotic compound, i.e., quercetin. For practical reasons we performed these metabolism experiments

on cells just reaching confluence after growing for different periods in 6-well plastic trays in the presence of 1 μ M quercetin (see Materials and Methods). Under these conditions the cells display a much more intense methyltransferase activity than when examined after reaching confluence and differentiation on Transwell® septa. This activity was not affected by quercetin (Fig. 4). On the other hand, sulfotransferase activity, nearly absent to start with, was progressively re-expressed within 6 days (Fig. 4). After 9 days metabolic activity was maintained at approximately the same level as after 6 days of growth, but signs of a cytotoxic effect of the polyphenol appeared (not shown).

Discussion

Caco-2 are a widespread model for transepithelial transport. If they are to provide reliable indications as to the absorption of a compound in the gastrointestinal tract they must correctly reflect metabolic as well as transport activity. Our experience with these cells was however that they can exhibit extremely variable phase II metabolic activity on a good substrate such as quercetin. Differences in metabolic capabilities can lead to discrepant results. For example in studying the transport of quercetin and its glycosides across Caco-2 monolayers a group [29] reported a rapid passage of unmodified quercetin, while others [28, 30] found conjugation products on both sides of the monolayer. Assessment of intestinal absorption *in vivo* is complicated by metabolic transformations taking place at other sites, chiefly the liver, but experiments isolating the absorption step by using mounts of the intestinal wall or explanted intestine segments [31, 32] indicate that conjugative metabolism ought to be a feature of any reliable absorption model for polyphenols. If Caco-2 cells are to be used as such a model, they ought therefore to be metabolically active. Furthermore a standardization of the metabolic properties of the cells would be of great help in comparing results obtained from different laboratories.

Our results indicate that the expression of conjugating enzymes can be downregulated by prolonged culture under standard conditions or by “traumatic” events such as freezing followed by recovery and expansion of, perhaps, a selected population. Xenobiotic compounds can induce the expression of detoxifying enzymes in Caco-2 cells (e.g. [33, 34]). Quercetin is known to increase the expression of claudin-4, a protein of the tight junctions in Caco-2 cells lowering the permeability of a monolayer to markers of pericellular permeation [35]. We found that culture of these cells with a low concentration of the intended transport/metabolism substrate, i.e., quercetin, succeeded in re-establishing expression of the relevant metabolising enzymes, i.e. sulfotransferases. We suggest therefore that Caco-2 cells to be employed in transport and/or metabolism studies be routinely “preactivated” by culture for a few days in the presence of low concentrations of the compounds of interest.

Heterogeneity of metabolic activity is probably a widespread characteristic of cultured cells, as suggested also by preliminary experiments with HepG2 cells (Biasutto, L. et al., unpublished). Inducibility of metabolic enzymes has been shown with different cells lines [26, 36]. “Preactivation” may represent a general strategy to restore metabolic homogeneity between different clones of the same cell line. Metabolic activation and standardization may be relevant also for Phase I metabolism, since various natural occurring flavonoids have been shown to modulate also specific cytochrome P450 isozymes [37-39].

Acknowledgements

We thank E. Papini, M. De Bernard, I. Dell’Aica for cells, N. Sassi for help with some experiments. This work was supported in part by grants from the Italian Association for Cancer Research (AIRC) and the Italian Foundation for Basic Research (FIRB). L.B. was supported by a fellowship of the Fondazione Cassa di Risparmio di Padova e Rovigo.

References

- 1 Meunier V, Bourrié M, Berger Y, Fabre G: The human intestinal epithelial cell line Caco-2; pharmacological and pharmacokinetic applications. *Cell Biol Toxicol* 1995;11:187-194.
- 2 Van Breemen RB, Li Y: Caco-2 cell permeability assays to measure drug absorption. *Expert Opin Drug Metab Toxicol* 2005;1:175-185.
- 3 Van de Kerkhof EG, de Graaf IA, Groothuis GM: In vitro methods to study intestinal drug metabolism. *Curr Drug Metab* 2007;8:658-675.

- 4 Sun H, Chow EC, Liu S, Du Y, Pang KS: The Caco-2 cell monolayer: usefulness and limitations. *Expert Opin Drug Metab Toxicol* 2008;4:395-411.
- 5 Gregory PA, Lewinsky RH, Gardner-Stephen DA, Mackenzie PI: Coordinate regulation of the human UDP-glucuronosyltransferase 1A8, 1A9, and 1A10 genes by hepatocyte nuclear factor 1 α and the caudal-related homeodomain protein 2. *Mol Pharmacol* 2004;65:953-963.
- 6 Liu X, Tam VH, Hu M: Disposition of flavonoids via enteric recycling: determination of the UDP-glucuronosyltransferase isoforms responsible for the metabolism of flavonoids in intact Caco-2 TC7 cells using siRNA. *Mol Pharm* 2007;4:873-882.
- 7 Tamura HO, Taniguchi K, Hayashi E, Hiyoshi Y, Nagai F: Expression profiling of sulfotransferases in human cell lines derived from extra-hepatic tissues. *Biol Pharm Bull* 2001;24:1258-1262.
- 8 Chen Y, Huang C, Zhou T, Chen G: Genistein Induction of Human Sulfotransferases in HepG2 and Caco-2 Cells. *Basic Clin Pharmacol Toxicol* 2008;103:553-559.
- 9 Meinel W, Ebert B, Glatt H, Lampen A: Sulfotransferase forms expressed in human intestinal Caco-2 and TC7 cells at varying stages of differentiation and role in benzo[a]pyrene metabolism. *Drug Metab Dispos* 2008;36:276-283.
- 10 Zhang L, Zheng Y, Chow MS, Zuo Z: Investigation of intestinal absorption and disposition of green tea catechins by Caco-2 monolayer model. *Int J Pharm* 2004;287:1-12.
- 11 Poquet L, Clifford MN, Williamson G: Investigation of the metabolic fate of dihydrocaffeic acid. *Biochem Pharmacol* 2008;75:1218-1229.
- 12 Fisher MB, Labissiere G: The role of the intestine in drug metabolism and pharmacokinetics: an industry perspective. *Curr Drug Metab* 2007;8:694-699.
- 13 Benet LZ, Cummins CL: The drug efflux-metabolism alliance: biochemical aspects. *Adv Drug Deliv Rev* 2001;50 Suppl 1:S3-S11.
- 14 Benet LZ, Cummins CL, Wu CY: Unmasking the dynamic interplay between efflux transporters and metabolic enzymes. *Int J Pharm* 2004;277:3-9.
- 15 Silberberg M, Morand C, Mathevon T, Besson C, Manach C, Scalbert A, Remesy C: The bioavailability of polyphenols is highly governed by the capacity of the intestine and of the liver to secrete conjugated metabolites. *Eur J Nutr* 2006;45:88-96.
- 16 Crespy V, Morand C, Manach C, Besson C, Demigne C, Remesy C: Part of the quercetin absorbed in the small intestine is conjugated and further secreted in the intestinal lumen. *Am J Physiol* 1999;277:G120-G126.
- 17 O'Leary KA, Day AJ, Needs PW, Mellon FA, O'Brien NM, Williamson G: Metabolism of quercetin-7- and quercetin-3-glucuronides by an in vitro hepatic model: the role of human β -glucuronidase, sulfotransferase, catechol-O-methyltransferase and multi-resistant protein 2 (MRP2) in flavonoid metabolism. *Biochem Pharmacol* 2003;65:479-491.
- 18 Akao T, Sakashita Y, Hanada M, Goto H, Shimada Y, Terasawa K: Enteric excretion of baicalin, a flavone of *Scutellariae Radix*, via glucuronidation in rat: involvement of multidrug resistance-associated protein-2. *Pharm Res* 2004;21:2120-2126.
- 19 Akao T, Hanada M, Sakashita Y, Sato K, Morita M, Imanaka T: Efflux of baicalin, a flavone glucuronide of *Scutellariae Radix*, on Caco-2 cells through multidrug resistance-associated protein 2. *J Pharm Pharmacol* 2007;59:87-93.
- 20 Williamson G, Aeberli I, Miguet L, Zhang Z, Sanchez MB, Crespy V, Barron D, Needs P, Kroon PA, Glavinas H, Krajcsi P, Grigorov M: Interaction of positional isomers of quercetin glucuronides with the transporter ABCC2 (cMOAT, MRP2). *Drug Metab Dispos* 2007;35:1262-1268.
- 21 Lambert JD, Sang S, Lu AY, Yang CS: Metabolism of dietary polyphenols and possible interactions with drugs. *Curr Drug Metab* 2007;8:499-507.
- 22 Maier-Salamon A, Hagenauer B, Reznicek G, Szekeres T, Thalhammer T, Jäger W: Metabolism and disposition of resveratrol in the isolated perfused rat liver: role of Mrp2 in the biliary excretion of glucuronides. *J Pharm Sci* 2008;97:1615-1628.
- 23 Volpe DA: Variability in Caco-2 and MDCK cell-based intestinal permeability assays. *J Pharm Sci* 2008;97:712-725.
- 24 Salvini S, Charbonnier M, Defoort C, Alquier C, Lairon D: Functional characterization of three clones of the human intestinal Caco-2 cell line for dietary lipid processing. *Br J Nutr* 2002;87:211-217.
- 25 Sambuy Y, De Angelis I, Ranaldi G, Scarino ML, Stamatii A, Zucco F: The Caco-2 cell line as a model of the intestinal barrier: influence of cell and culture-related factors on Caco-2 cell functional characteristics. *Cell Biol Toxicol* 2005;21:1-26.
- 26 Lançon A, Hanet N, Jannin B, Delmas D, Heydel JM, Lizard G, Chagnon MC, Artur Y, Latruffe N: Resveratrol in human hepatoma HepG2 cells: metabolism and inducibility of detoxifying enzymes. *Drug Metab Dispos* 2007;35:699-703.
- 27 Hebbar V, Shen G, Hu R, Kim BR, Chen C, Korytko PJ, Crowell JA, Levine BS, Kong AN: Toxicogenomics of resveratrol in rat liver. *Life Sci* 2005;76:2299-2314.
- 28 Biasutto L, Marotta E, De Marchi U, Zoratti M, Paradisi C: Ester-based precursors to increase the bioavailability of quercetin. *J Med Chem* 2007;50:241-253.
- 29 Walgren RA, Walle UK, Walle T: Transport of quercetin and its glucosides across human intestinal epithelial Caco-2 cells. *Biochem Pharmacol* 1998;55:1721-1727.
- 30 Murota K, Shimizu S, Chujo H, Moon JH, Terao J: Efficiency of absorption and metabolic conversion of quercetin and its glucosides in human intestinal cell line Caco-2. *Arch Biochem Biophys* 2000;384:391-397.
- 31 Crespy V, Morand C, Besson C, Manach C, Démigné C, Rémésy C: Comparison of the intestinal absorption of quercetin, phloretin and their glucosides in rats. *J Nutr* 2001;131:2109-2114.
- 32 Dragoni S, Gee J, Bennett R, Valoti M, Sgaragli G: red wine alcohol promotes quercetin absorption and directs its metabolism towards isorhamnetin and tamarixetin in rat intestine in vitro. *Br J Pharmacol* 2006;147:765-771.
- 33 Lampen A, Ebert B, Stumkat L, Jacob J, Seidel A: Induction of gene expression of xenobiotic metabolism enzymes and ABC-transport proteins by PAH and a reconstituted PAH mixture in human Caco-2 cells. *Biochim Biophys Acta* 2004;1681:38-46.
- 34 Xu C, Li CY, Kong AN: Induction of phase I, II and III drug metabolism/transport by xenobiotics. *Arch Pharm Res* 2005;28:249-268.
- 35 Amasheh M, Schlichter S, Amasheh S, Mankertz J, Zeitz M, Fromm M, Schulzke JD: Quercetin enhances epithelial barrier function and increases claudin-4 expression in caco-2 cells. *J Nutr* 2008;138:1067-1073.
- 36 Sun XY, Plouzek CA, Henry JP, Wang TT, Phang JM: Increased UDP-glucuronosyltransferase activity and decreased prostate specific antigen production by benchanin A in prostate cancer cells. *Cancer Res* 1998;58:2379-2384.
- 37 Wood AW, Smith DS, Chang RL, Huang MT, Conney AH: Effects of flavonoids on the metabolism of xenobiotics. *Progr Clin Biol Res* 1986;213:195-210.
- 38 Ho PC, Saville DJ, Wanwimolruk S: Inhibition of human CYP3A4 activity by grapefruit flavonoids, furanocoumarins and related compounds. *J Pharm Pharm Sci* 2001;4:217-227.
- 39 Ciolino HP, Daschner PJ, Yeh GC: Dietary flavonols quercetin and kaempferol are ligands of the aryl hydrocarbon receptor that affect CYP1A1 transcription differentially. *Biochem J* 1999;715-722.