Eur J Nutr (2010) 49:373–384
DOI 10.1007/s00394-010-0095-x

ORIGINAL CONTRIBUTION

In vitro interactions between aged garlic extract and drugs used for the treatment of cardiovascular and diabetic patients

Katja Berginc · Simon Žakelj · Albin Kristl

Received: 26 July 2009 / Accepted: 21 January 2010 / Published online: 7 February 2010

Abstract
Background Disease preventing effects gained by garlic consumption have been recognized since early period of history, making commercially available garlic supplements attractive to the general public. Possible pharmacokinetic interactions which could occur between applied drugs and aged garlic extract (AGE) are unknown.
Aim To test in vitro impact of some garlic phytochemicals on P-glycoprotein (Pgp), the most recognized efflux transporter, and the effect of AGE on passive membrane permeability, absorptive and secretory intestinal transporters.
Methods Rat small intestine and Caco-2 cell monolayers, mounted in side-by-side diffusion chambers were used.
Results Hydrophilic sulphur compounds increased Pgp mediated Rhodamine 123 (Rho123) efflux, whereas the lipophilic ones increased Pgp efflux through rat ileum but not through Caco-2 cell monolayers. Increased activities of secretory (Pgp, multidrug-resistance associated protein 2) and absorptive (monocarboxylate transporter 1, organic anion transporting polypeptide) transporters involved in drug absorption were observed in rat small intestine and Caco-2 cell monolayers in the presence of AGE. Transport of drugs mediated by breast cancer resistance protein and H^+-oligopeptide transporter 1 was activated in rat intestine but inhibited through Caco-2 cells. Passive membrane permeability of tested compounds remained unaltered through rat small intestine, while significant changes were observed with Caco-2 cell monolayers.
Conclusions Due to the observed in vitro pharmacokinetic interactions between AGE and investigated cardiovascular, antidiabetic and antiviral drugs, in vivo absorption changes are possible, but the magnitude of change depends on the most profound process involved (influx, efflux, passive diffusion) in compounds permeability.

Keywords Aged garlic extract · Drugs · Interactions · Intestinal transporters · Rat intestine · Caco-2 cell monolayers

K. Berginc (✉) · S. Žakelj · A. Kristl
Faculty of Pharmacy, University of Ljubljana, Aškerčeva 7, p.p. 311, 1000 Ljubljana, Slovenia
e-mail: katja.berginc@ffa.uni-lj.si

Introduction

The health benefits acquired by garlic consumption have been recognized since early period of history [1]. The pharmacological activities (i.e. antihypertensive, lipid-lowering, anti-atherogenic, antitumorigenic, fibrinolytic, antioxidant, anticarcinogenic, immunomodulatory, antimicrobial, antihepatotoxic, hypogliceamic and other effects) of various garlic constituents [2] and garlic preparations [3] have been confirmed in numerous epidemiological human studies, preclinical animal studies and extensive in vitro research [3, 4]. The general belief that over-the-counter products are safe and lack adverse effects contributes to constantly rising simultaneous consumption of dietary supplements with or even instead of prescribed drugs [5]. Since herbal preparations represent a mixture of different and pharmacologically active phytochemicals, possible pharmacokinetic and/or pharmacodynamic interactions and consequently adverse reactions might be clinically manifested. In case of garlic, interactions have already been documented for administration of garlic with warfarin, ritonavir and saquinavir [6].

Because oral drug administration is the most popular one, altered intestinal transporter-enzyme interplay and passive membrane permeability caused by concomitant intake of garlic supplements may demonstrate themselves as higher or lower plasma drug concentrations, altered first-pass metabolite profile and gut and/or liver clearances. However, high intestinal concentrations of biopharmaceutical classification system (BCS) [7] Class I compounds (high solubility, high permeability) will saturate intestinal enzymes and transporters causing the interactions between these substances and herbal compounds clinically unimportant. On the contrary, many of BCS Class II, III and IV actively transported drugs are either low permeable or/and low soluble and are subjected to intestinal secretory (Class II, III) or absorptive (Class III, IV) transport processes and intestinal first-pass metabolism with Phase I and II enzymes. In general food coadministration can induce physiological changes in gastrointestinal tract; i.e. it modulates solubility profiles of compounds, affects the activity of secretory and absorptive intestinal transporters and enzymes [8]. Garlic phytochemicals, contained in herbal dietary supplements, might also influence some of the biochemical processes in the intestine beside having beneficial effects, since they have been shown to modulate enzyme [9] and transporter activities [P-glycoprotein (Pgp), multidrug-resistance associated protein (MRP2)] [8]. These effects could lead to significant pharmacokinetic plasma profile changes of the drug and possibly also to the pharmacodynamic interactions. Namely, different active principles contained in garlic supplements when absorbed, have already been proven to exert several pharmacological activities [3, 4].

The aim of this study was to screen and clarify the possible pharmacokinetic interactions which could occur between drugs and aged garlic extract (AGE) or its components at the site of absorption. Different compounds prescribed for the treatment of cardiovascular, diabetic conditions and infections were chosen, since garlic supplements are used concomitantly in all these situations due to their remedial properties. The selected drugs are also substrates for different intestinal transport systems, enabling us to evaluate the influence of AGE on absorptive [monocarboxylate transporter 1 (MCT1), H^+-dependent peptide cotransport system 1 (PepT1), organic anion transporting polypeptide (Oatp)] and secretory transporters [Pgp, MRP-2, breast cancer resistance protein (BCRP), organic cation transporter 1 (OCT1)] as well as on the passive membrane permeability (paracellular and transcellular). The drug permeability characteristics were assessed through different segments of the rat intestine and through Caco-2 cell monolayers using side-by-side diffusion chambers.

Materials and methods

Materials

Rhodamine 123 (Rho123), verapamil (Ver), benzbromarone (BB), digoxin, prazosin, furosemide, hydrochlorothiazide, atenolol, valsartan, ciprofloxacin, valacyclovir, glibenclamide, pravastatin, quinidine, adenosine, β-carotene, diallyl sulphide (DAS), diallyl disulfide (DADS), allyl methyl sulphide (AMS), methyl diallyl sulphide (MDS), *p*-cymene, *S*- and *R*-limonene, ferrulic acid (FE), quercetin (Q), amoxicilin (Amox), α-ciannohydroxycinnamic acid (CHC), 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid (DIDS) and salts for incubation salines were from Sigma-Aldrich Chemie (Deisenhofen, Germany). Losartan, sildenafil, atorvastatin, rosuvastatin, glipizide, sildenafil were purchased at Sequoia Research Products. *S*-allyl L-cysteine (SAC) standard was from TCI Europe NV (Zwijndrech, Belgium). All chemicals used in this study were of highest grade available. Kyolic® liquid AGE was produced by Wakanuga of America Co., Ltd (Mission Viejo, CA, USA), lot number 5H04A.

AGE composition

AGE is prepared by 20 months soaking process of sliced raw garlic in 5–20% aqueous ethanol at room temperature. During this time, lipid-soluble allicin-derived substances (mono-, di- and trisulfides, capaenes, dithiins and ajoenes [10, 11]) degrade. Because these are garlic's odiferous, irritating and oxidizing ingredients responsible for unwanted side effects, their presence in garlic supplements is undesirable although recent reports indicated their important pharmacological activities [4, 12]. AGE therefore consists mostly of water soluble sulfur constituents (*S*-allyl cisteine, *S*-allyl mercaptocysteine, derivatives of γ-glutamyl *S*-allyl cisteine) [5] and only minute quantities of lipid soluble volatives (DADS, DAS, DATS) [13]. Since aminoacids, proteins, lipids, vitamins, minerals, prostaglandins, flavonoids and flavons, polysaccharides, saponins, steroids, selenium organic derivatives can also be found in raw garlic, these compounds could also be present in the final garlic product—AGE but at different concentrations than the ones in raw garlic [3].

AGE used in this study was standardized to SAC (1.27 g/L).

In vitro transport studies across Caco-2 cell monolayers and rat small intestine

Caco-2 cells were obtained from American Tissue Culture Collection (ATCC) HTB.37, lot 2463681 and were grown on Transwell or Snapwell Costar culture inserts with a

polycarbonate membrane (diameter 12 mm and pore size 0.4 μm). 100,000 cells/filter membrane were used for seeding and the medium was changed every two days. At Day 15, transepithelial electrical resistance (TEER) was measured for each filter with Caco-2 cell monolayers. If the TEER values were in the range of 450–750 Ωcm^2, the Caco-2 cell monolayers were used for the subsequent testing of permeability at Day 21.

The Caco-2 cells grown on Snapwell Costar culture inserts were carefully rinsed with Ringer buffer and then placed between two compartments of EasyMount side-by-side diffusion chambers (Physiologic Instruments, San Diego, USA). 2.5 mL of bathing solution (Ringer buffer) on each side of the Caco-2 cell monolayer was maintained at 37 °C and continuously oxygenated and circulated by bubbling with carbogen (95% O_2, 5% CO_2). 10 mM glucose and 10 mM mannitol were always added to the basolateral (BL) and apical (AP) sides, respectively. After 25 min of preincubation, 0.5 mL of the concentrated solution containing investigated substances was added to the AP side (if studying apical-to-basolateral (AP–BL) transport) or the BL side (if studying basolateral-to-apical (BL–AP) transport). The drugs studied were usually used in 100 μmol/L donor concentrations. Exceptions due to low solubility or high toxicity are designated in the text or tables. Two hundred and fifty microliters of samples were withdrawn from the acceptor side every 15 min up to 75 min, and replaced each time by fresh Ringer buffer containing all necessary ingredients at appropriate concentrations. In the second phase of the experiment, AGE concentrate was added to the AP side of Caco-2 cell monolayers to give 1% (*v*/*v*) final concentration of AGE. The sampling continued every 15 min up to 150 min. The withdrawn samples were replaced by fresh Ringer buffer with all necessary ingredients. In the third phase of the experiment, the appropriate specific transporter inhibitor was added to the AP side and the experiment continued for an additional 75 min with 15 min sampling periods (Pgp inhibitor verapamil 200 μmol/L, MRP-2 inhibitor benzbromarone 50 μmol/L, MCT1 inhibitor α-ciannohydroxycinnamic acid 1 mmol/L, OATP inhibitor 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid 1 mmol/L, PepT1 inhibitor amoxicillin Amox 10 mmol/L).

Only those Caco-2 cell monolayers with TEER values that remained constant during the whole experiment were used.

The experiments conducted with rat intestinal tissue conform to the Law for the Protection of Animals (Republic of Slovenia) and were registered at the Veterinary Administration of the Republic of Slovenia. Rat small intestine was obtained from male Wistar rats (250–320 g) fasted 18 h prior to the experiments. After killing and laparotomy, the intestine was rinsed with ice-cold 10 mM glucose Ringer solution. Duodenum, located 25 cm proximally to the pyloric sphincter, jejunum, located 25–60 cm distally from the pyloric sphincter, and ileum, 20 cm proximally to the ileo-caecal junction, were used for the experiments. The intestinal tissue was cut into 3 cm long segments, excluding visible Peyer's patches. These intestinal segments were opened along the mesenteric border, stretched onto inserts with an exposed tissue area of 1 cm^2 and then placed between two compartments of EasyMount side-by-side diffusion chambers (Physiologic Instruments, San Diego, USA). The experimental procedure continued in the same manner as described for Caco-2 cell monolayers.

Electrical measurements

The diffusion chambers were equipped with two pairs of Ag/AgCl electrodes for measuring transepithelial potential difference (PD) and short circuit current (I_{sc}) with a multi channel voltage-current clamp (model VCC MC6, Physiologic Instruments). The viability and integrity of tissue and Caco-2 cells were checked by monitoring PD, I_{sc} and TEER every 15 min during the experiments. In viable rat tissues and Caco-2 cells, TEER did not change statistically significantly over 150 or 225 min periods. The average TEER from 15 min to 150 or 225 min was calculated and also used for evaluating the tissue/cell integrity and viability. The viability of rat small intestine was additionally checked by recording the increase of I_{sc} and PD after the addition of stock glucose solution to the mucosal compartment at the end of experiment (final glucose concentration was 25 mM). Tissue segments were considered viable if the PD value after the addition of glucose was lower than −1.0 mV and if the average TEER values recorded during the experiment were between 20 and 40 Ωcm^2. The Caco-2 cell culture monolayers were considered viable if the average TEER values between 15 and 150 or 225 min were in the range of 500–800 Ωcm^2, and if they exhibited a PD lower than −0.5 mV during the experiment.

Data analysis

The apparent permeability coefficient (P_{app}) was calculated according to Eq. (1):

$$P_{app} = \frac{dc}{dt}\frac{V}{c_0 A} \quad (1)$$

where *dc*/*dt* is the change in concentration of the examined substance in the acceptor compartment per unit time under steady state conditions, *V* is the volume of the acceptor compartment, *A* the exposed surface area (1 cm^2 for rat jejunum and 1.13 cm^2 for Caco-2 cell monolayers) and c_0

the initial concentration of the examined substance in the donor solution.

When testing the influence of individual garlic phytochemicals on Pgp-mediated Rho123 efflux, values R were calculated according to Eq. (2) and (3):

$$R = \frac{R_{Phy}}{R_{Rf}} \tag{2}$$

$$R_{\mathrm{Phy}} = \frac{P_{\mathrm{app}}(B-A)^{\mathrm{Phy}}}{P_{\mathrm{app}}(A-B)^{\mathrm{Phy}}}$$
$$R_{\mathrm{Rf}} = \frac{P_{\mathrm{app}}(B-A)^{\mathrm{Rf}}}{P_{\mathrm{app}}(A-B)^{\mathrm{Rf}}} \tag{3}$$

where R_{Phy} and R_{Rf} represent the efflux ratio for Rho123 determined in the presence of phytochemicals (Phy) or in their absence (reference—Rf). $P_{\mathrm{app}}(B-A)^{\mathrm{Phy}}$ and $P_{\mathrm{app}}(A-B)^{\mathrm{Phy}}$ represent the apparent permeability of Rho123 determined in *S–M/BL–AP* (*BA*) and *M–S/BL–AP* ($A-B$) directions in the presence of tested phytochemical (Phy). $P_{\mathrm{app}}(B-A)^{\mathrm{Rf}}$ and $P_{\mathrm{app}}(A-B)^{\mathrm{Rf}}$ are apparent permeabilities of Rho123 determined in S–M/BL–AP and M–S/AP–BL directions without phytochemicals present.

Results in tables are presented as means ± SD of at least three measurements. Data were evaluated statistically using SPSS 16.0 for Windows. Where appropriate, *F* test for testing the equality of variances and, afterwards, two-tailed Student *t* test ($\alpha = 0.05$), were used. Otherwise, one-way ANOVA, followed by Bonferroni post-hoc test were applied.

HPLC parameters

Concentrations of FLU and Rho123 were measured with a fluorescence detector Tecan GENious ($\lambda_{ex} = 485$ nm, $\lambda_{em} = 535$ nm). One hundred microliters of a sample was diluted with 100 µL of 0.025 M NaOH to measure the concentration of FLU and with 100 µL of 0.01 M HCl for Rho123. Other drugs were analyzed by HPLC (all components: binary pump, well-plate sampler, column thermostat and diode-array UV detector were Series 1100 from AgilentTechnologies, Waldbronn, Germany).

Results

The impact of different garlic phytochemicals on Pgp activity

We tested the lipophilic compounds DADS, DAS, MDS and their metabolites, formed in vivo after garlic consumption and detectable in the exhaled breath AMS, *R*-/*S*-limonen and *p*-cymen (Table 1, 2) [14]. All these substances were applied in 20 µmol/L concentrations. SAC, the main water soluble sulfur compound in AGE, was also assessed since it is well absorbed [15] and it is a pharmacologically active ingredient with hepatoprotective, hypolipidaemic, antioxidant and anticancer activities [4, 15]. SAC was tested at 20 (↓c) and 60 (↑c) µmol/L, the latter concentration reflecting the AGE SAC content that this commercially available product was standardized to [13]. Flavonoid quercetin, a known Pgp inhibitor [16], was also applied in two different concentrations: the high one (↑c 125 µmol/L) and the low one (↓c 5 µmol/L). Other examined substances (adenosine, *β*-carotene and FE) were evaluated at 20 µmol/L.

Significantly enhanced Pgp mediated Rho123 efflux through the rat ileum was observed in the presence of DADS, MDS, AMS, SAC (20 and 60 µmol/L) and Q (5 µmol/L). It is known that Q binds to the H-binding site of Pgp leaving other three Pgp binding/regulatory sites unoccupied. Since Rho123 transport is mediated by the *R*-site of Pgp, lower Q concentrations (i.e. 5 µmol/L) might induce allosteric changes favoring Rho123 efflux whereas higher (i.e. 125 µmol/L) Q concentrations nonspecifically occupy not just *H*- but also *R*-site resulting in significantly lower Rho123 efflux as predicted by the theory of concentration dependent and positive-cooperative manner [13] of Pgp action. Therefore 125 µmol/L Q inhibited Rho123 efflux, which is in accordance with literature data that Q is a Pgp inhibitor [12] and sometimes even an enhancer [17]. The other tested substances had no significant impact on Pgp mediated Rho123 efflux through the rat intestine (Table 2).

Phytochemicals that significantly affected Pgp in the rat ileum, were further investigated on Transwell grown Caco-2 cell monolayers. Interestingly, DADS, AMD, MDS caused no significant changes of Rho123 efflux through Caco-2 cell monolayers. Similar to the effects on the rat ileum, SAC (both concentrations) and Q (only low concentration) caused increase of Rho123 efflux compared to the reference values for Caco-2 cells, but the effect was insignificant. Q at higher concentration tested did not inhibit Pgp efflux. In fact a significant increase of Pgp mediated efflux was observed. Even higher concentrations of Q might be needed to cause Pgp inhibition in these cells.

Drug passive membrane permeability in the presence of 1 *v*/*v*% mucosal/apical AGE

Passive membrane permeability in M–S/AP–BL direction was assessed for drugs that are not subjected to active uptake when Ringer buffer pH 7.4 is used on both sides of intestine or Caco-2 cell monolayers. These compounds are also presented in the Table 3 and were selected to represent all BCS classes with a broad range of fraction of dose absorbed (F_{abs}). Where F_{abs} could not be obtained in the literature, bioavailability (BU) is given instead. The M–S

Table 1 HPLC conditions for measuring the concentrations of tested drugs

Compound	Col[a]	T (°C)	FR (ml/min)	A:B[b]	pH	t_r (min)	λ_{max} (nm)
Digoxin	B	30	2.5	73:27	3.0	2.3	220
Prazosin	A	30	1.5	81.5:18.5	3.0	1.7	254
Furosemide	B	35	1.7	77:23	3.0	3.1	234
Hydrochlorthiazide	C	25	0.7	85:15	3.0	3.7	225
Atenolol	C	25	0.7	83:17	7.0	3.2	225
Losartan	B	50	1.6	74:26	7.0	2.5	254
Valsartan	B	30	2.7	70:30	2.8	2.6	205
Sildenafil	A	35	2	$70:30 \xrightarrow[1.8\text{min}]{} 50:50$	3.0	1.7	225
Atorvastatin	D	35	3	55:45	4.0	0.5	244
Pravastatin	A	30	1.6	73:27	3.0	1.9	237
Rosuvastatin	A	35	2	60:40	3.0	1.8	245
Glipizide	B	50	2.5	74:26	3.0	1.5	230
Glibenclamide	B	50	3	70:30	3.0	4.1	230
Ciprofloxacin	A	35	2.0	$84:16 \xrightarrow[4.9\text{min}]{} 60:40$	3.9	3.6	278
Valacyclovir[c]	D	35	2.0	$99:1 \xrightarrow[2.5\text{min}]{} 84:16$	6.3	4.1	254

Column type (*Col*), column temperature (*T*), flow rate (*FR*), composition of the mobile phase (*A:B*), aqueous phase(*A*), pH of the phosphate buffer in the mobile phase, retention time t_r, and detection wavelength λ_{max})

[a] Columns, designated with letters *A* (Phenomenex Onyx Monolythic C18 100 × 3.0 mm), *B* (Phenomenex Onyx Monolythic C18 25 × 4.6 mm), *C* (Synergie 2.5 μ Polar RP 100A, 100 × 3.0 mm), *D* (Chromolith RP 8, 100 × 4.6 nm) were used

[b] The organic phase (*B*) was acetonitrile in all cases except for ciprofloxacin and valacyclovir, where methanol was used instead. Gradient conditions and the time, at which the conditions changed, are shown

[c] The concentration of valacyclovir was determined as the sum of the measured concentrations of valacyclovir and its metabolite acyclovir (t_r = 2.0 min)

permeabilities determined through the rat small intestine did not change significantly in the presence of AGE compared to reference values (Tables 4, 5, 6) for the majority of the drugs tested (prazosin, furosemide, hydrochlorothiazide, atenolol, losartan, valsartan, sildenafil, atorvastatin, rosuvastatin, ciprofloxacin). However, M–S permeabilities significantly increased for antidiabetic agents glipizide, glibenclamide and for the hypolipidaemic drug pravastatin through the rat intestine after the addition of AGE. Interestingly, AP–BL permeabilities of all tested compounds mentioned above through Caco-2 cell monolayers (Tables 4, 5, 6) were altered significantly by the presence of AGE except of furosemide, a low permeable Class IV BCS compound [18].

Drug efflux in the presence of 1 *v/v*% mucosal/apical AGE

To evaluate the impact of AGE on the intestinal secretory transporters, structurally different drugs utilized for the management of cardiovascular, diabetic and viral conditions were selected as presented in Table 3. According to the literature and/or our results with specific secretory inhibitors, hydrochlorothiazide, digoxin [19], glibenclamid but not glipizide are Pgp substrates, whereas losartan [20], valsartan [21], furosemide [22], atorvastatin [23] and pravastatin [23] are secreted into the intestinal lumen by Pgp and MRP-2. Prazosin is a substrate for Pgp [24] and BCRP [25], while the transport of rosuvastatin and ciprofloxacin is mediated by BCRP [23] and OCT1 [26], respectively. Atenolol [27], a low permeability marker, and sildenafil are not actively transported through intestinal tissue. The asymmetrical permeabilities (i.e. higher S–M/BL–AP P_{app} than M–S/BL–AP P_{app} values) of selected drugs were first evaluated in the "reference phase" of the two- or three-phase experiments without AGE (data in the "Ref" column in the Tables 4, 5, 6) through Caco-2 cell monolayers and rat ileum [28] (for drugs that are Pgp, OCT1 and BCRP substrates) or rat duodenum [28] (for MRP-2 substrates). Using an appropriate inhibitor (verapamil for Pgp, benzbromarone for MRP-2 or both for mixed substrates and quinidine for OCT1) the AGE influence on secretory transporters involved in the absorption process of tested drugs was additionally verified.

The influence of 1 *v/v*% AGE on the permeability of Pgp substrates

Permeabilities of Pgp substrates (hydrochlorothiazide, digoxin, glibenclamide) were determined in the rat ileum and Caco-2 cell monolayers. For all Pgp substrates tested, significantly higher S–M/BL–AP P_{app} than M–S/AP–BL

P_{app} values were obtained, confirming Pgp mediated active transport involved in the absorption of these compounds (Table 4). The addition of AGE to the M side of the rat ileum increased the S–M P_{app} values, but the changes were not statistically significant. We could not determine the permeability of digoxin through the rat ileum because it is extensively metabolised in the rat small intestine. However, digoxin metabolism in in vivo human intestine and in in vitro Caco-2 cell monolayers is negligible [7], therefore the experiments with this drug were performed only through Caco-2 cell monolayers. The results determined through Caco-2 cell monolayers showed significantly increased Pgp mediated transport in the presence of AGE for digoxin and hydrochlorothiazide. Because digoxin is a drug with narrow therapeutic window, significantly increased Pgp mediated efflux of lower therapeutic doses in nmol/L range might be even more pronounced. The glibenclamide BL–AP permeability through Caco-2 cells contrary to the other Pgp substrates tested significantly decreased.

Table 2 The influence of individual garlic phytochemicals on Rho123 efflux through the rat ileum and Transwell grown Caco-2 cell monolayers

Phytochemical	c (μM)	R values		S/NS	
		Rat ileum	Caco-2	Rat ileum	Caco-2
Q (↓c)	5	2.1 ± 0.2	1.3 ± 0.1	S	NS
Q (↑c)	125	0.6 ± 0.2	1.9 ± 0.1	S	S
SAC (↓c)	20	3.1 ± 0.2	1.3 ± 0.2	S	NS
SAC (↑c)	60	1.3 ± 0.1	1.5 ± 0.2	S	NS
DAS	5	1.0 ± 0.2		NS	
DADS	20	2.0 ± 0.2	1.2 ± 0.2	S	NS
MDS	20	1.9 ± 0.1	1.2 ± 0.2	S	NS
AMS	20	2.7 ± 0.2	1.0 ± 0.1	S	NS
FE	20	0.7 ± 0.3		NS	
R+ limonen	20	0.8 ± 0.3		NS	
S-limonen	20	1.0 ± 0.2		NS	
p-cymen	20	0.9 ± 0.2		NS	
b-caroten	20	1.1 ± 0.1		NS	
Adenosine	20	0.6 ± 0.2		NS	

The concentrations (*c*) of phytochemicals used during the experiments and R values are presented. The R values represent the ratio between S–M/BL–AP and M–S/AP–BL of Rho123 P_{app} values before and after the addition of the phytochemical to the M/AP incubation medium and were calculated according to the equation (2)

↓c, low concentration of Q (5 μM) and SAC (20 μM)

↑c, high concentration of Q (125 μM) and SAC (60 μM)

S/NS statistical analysis of R change, *S* significant ($p < 0.05$), *NS* not significant ($p > 0.05$)

The influence of 1 *v/v*% AGE on the permeability of mixed Pgp and MRP-2 substrates

Furosemide, losartan, valsartan, atorvastatin and pravastatin are subjected to intestinal Pgp and MRP-2 transport. To test the effect of AGE on both transporters and the contribution of each transporter to compounds secretion, the permeability experiments through Caco-2 cells and through ileum [28] (with the highest Pgp expression) and through duodenum [28] (with the highest MRP-2 expression) were conducted and afterwards in the third phase of the experiment the Pgp (Verapamil) and MRP-2 (Benzbromarone) inhibitors were added to M/AP side.

Table 3 List of the tested drugs, their theurapeutic indications, BCS classification, fraction of dose absorbed (F_{abs}) and bioavailability (*BU*) data from literature cited in the brackets. The donor concentrations (*c*) applied in this study are also presented

Substance	Therapeutic indications	BCS	F_{abs} (%)	BU (%)	c (μM)
Digoxin	Cardiac glycoside	II [7]	81 [43]		20
Prazosin	α-adreneric antagonist	I [39]	77–95 [43]		100
Furosemide	Diuretic	IV [7]	61 [43]		100
Sildenafil[a]	Erectile dysfunction		38 [44]		100
Hydrochlortiazide	Diuretic	III [7]	65–72 [43]		100
Atenolol[a]	β-adreneric antagonist	III [7]	50 [43]		100
Losartan	Angiotensin II antagonist			33 [41]	100
Valsartan	Angiotensin II antagonist	III [7]	23–39 [42]		100
Atorvastatin	HMG-CoA inhibitor	II [7]	12 [32]		100
Pravastatin	HMG-CoA inhibitor	III [7]	34 [43]		50
Rosuvastatin	HMG-CoA inhibitor			20 [33]	50
Glipizide	Antidiabetic	II [7]			100
Glibenclamide	Antidiabetic	II/IV [45]			100
Ciprofloxacin	Quinolon antibiotic	II/IV [7, 45]	> 69 [43]		50
Valacyclovir	Virostatic			15–30 [46]	100

[a] Only passively diffusing drugs

Table 4 Permeabilities of Pgp substrates through the rat ileum and Caco-2 cell monolayers determined in the reference (Ref) phase, after the addition of AGE to M/AP side in the second phase and in the presence of inhibitor (verapamil, Ver) in the third phase of the permeability experiments

Pgp substrates	P_{app} (*10^{-6} cm/s)						
		Ref		AGE		Inhibitor	
		M–S/AP–BL	S–M/BL–AP	M–S/AP–BL	S–M/BL–AP	M–S/AP–BL	S–M/BL–AP
Digoxin	Caco-2	7.1 ± 0.9	19.6 ± 1.6	11.0 ± 0.8*	37.4 ± 4.0*	10.6 ± 0.7	19.9 ± 6.1**
Hydrochlortiazide	Ileum	6.5 ± 2.4	12.5 ± 4.2	7.1 ± 1.6	19.5 ± 2.3*	8.8 ± 2.3	5.6 ± 1.2**
	Caco-2	0.6 ± 0.0	1.1 ± 0.3	0.8 ± 0.0*	3.7 ± 0.2*	0.8 ± 0.0	1.1 ± 0.6**
Glibenclamide	Ileum	1.8 ± 0.7	18.8 ± 3.4	2.8 ± 0.5*	22.2 ± 4.3	10.6 ± 1.3*	14.1 ± 1.0**
	Caco-2	18.1 ± 0.8	46.1 ± 7.5	21.8 ± 3.7	24.7 ± 6.7*	24.9 ± 5.7	25.4 ± 8.6

* Statistically significant changes of P_{app} determined in the presence of AGE compared to the reference (Ref) values of tested compound

** Statistically significant changes of P_{app} determined in the presence of Inhibitor compared to P_{app} values of tested compound in the presence of AGE

Table 5 Permeabilities of Pgp and MRP-2 substrates through the rat duodenum, rat ileum and Caco-2 cell monolayers determined in the reference (*Ref*) phase, after the addition of AGE to M/AP side in the second phase and in the presence of inhibitor (benzbromarone BB, verapamil Ver or both) in the third phase of the permeability experiments

Pgp and MRP2 substrates	P_{app} (*10^{-6} cm/s)							
		Ref		AGE		Inhibitor		
		M–S/AP–BL	S–M/BL–AP	M–S/AP–BL	S–M/BL–AP	Inhibitor	M–S/AP–BL	S–M/BL–AP
Losartan	Iileum	3.3 ± 1.0	15.5 ± 3.3	4.0 ± 0.4	23.8 ± 3.3*	BB	8.9 ± 2.6*	19.5 ± 1.7**
Valsartan	Duodenum	2.3 ± 0.1	9.0 ± 2.7	3.6 ± 1.7	10.6 ± 2.8	BB	4.6 ± 2.2	6.0 ± 0.6**
	Ileum	–	8.4 ± 2.3	–	11.2 ± 1.9	Ver	–	8.5 ± 1.3
	Caco-2	0.2 ± 0.0	0.7 ± 0.0	0.4 ± 0.0*	1.3 ± 0.0*	BB + Ver	0.4 ± 0.0	0.7 ± 0.1**
Furosemide	Duodenum	–	16.7 ± 3.9	–	18.9 ± 1.5	BB	–	5.1 ± 0.5**
	Ileum	2.8 ± 0.8	14.0 ± 3.4	2.3 ± 0.3	16.2 ± 2.1	Ver	2.6 ± 0.9	9.4 ± 2.7**
	Caco-2	0.3 ± 0.0	7.1 ± 0.6	0.4 ± 0.1	11.8 ± 0.8*	BB	0.5 ± 0.3	4.9 ± 0.9**
Atorvastatin	Duodenum	1.1 ± 0.2	12.0 ± 1.2	1.4 ± 0.3	16.2 ± 1.5*	BB	4.3 ± 0.3	9.4 ± 1.0**
	Ileum	0.9 ± 0.3	14.8 ± 0.8	1.0 ± 0.5	19.5 ± 3.1*	Ver	3.6 ± 0.4	8.7 ± 1.5**
	Caco-2	2.4 ± 0.1	4.4 ± 0.3	0.6 ± 0.3*	5.9 ± 0.0*	BB+Ver	3.4 ± 0.3*	3.9 ± 1.7
Pravastatin	Duodenum	3.5 ± 0.2	10.7 ± 2.8	4.7 ± 0.5*	13.7 ± 2.0	BB	7.2 ± 1.2*	5.8 ± 2.1**
	Ileum	–	12.3 ± 0.2	–	15.4 ± 1.2*	Ver	9.5 ± 2.3*	9.4 ± 2.8**

* Statistically significant changes of P_{app} determined in the presence of AGE compared to reference (Ref) values of tested compound

** Statistically significant changes of P_{app} determined in the presence of Inhibitor compared to P_{app} values of tested compound in the presence of AGE

– The permeability was not monitored during experiment

One can see from the results in Table 5 that the secretion of all tested substances increased after the addition of AGE to M/AP side of the rat small intestine or Caco-2 cell monolayers. The permeability significantly increased through Caco-2 cell monolayers for all examined compounds, for atorvastatin only through duodenum and through ileum for losartan, atorvastatin and pravastatin. However, in an attempt to separate the contribution of both transporters to drugs' permeability, inhibitors were added afterwards and the permeability decrease of each drug was further monitored. The results with BB and Ver clearly indicate that furosemide secretion is predominately mediated by MRP-2 with minor Pgp contribution since the addition of BB induced significant S–M permeability decrease, whereas no significant changes were observed in the presence of Ver. Valsartan permeability was tested through both rat ileum and duodenum. Because a significant S–M permeability decrease induced by benzbromarone was observed only in the duodenum whereas in the ileum there was no permeability change with Pgp inhibitor

Table 6 The influence of AGE on the permeability of other investigated compounds through the rat ileum or Caco-2 cell monolayers. The transporters responsible for the drugs′ efflux are also included in the table

Other	Transporters	P_{app} (*10^{-6} cm/s)				
			Ref		AGE	
			M–S/AP–BL	S–M/BL–AP	M–S/AP–BL	S–M/BL–AP
Prazosin	BCRP, Pgp	Ileum	7.3±1.8	22.2 ± 4.4	10.4 ± 2.9	34.7 ± 6.8
Rosuvastatin	BCRP	Ileum	3.3 ± 1.5	17.0 ± 1.6	2.9 ± 1.5	23.0 ± 1.3*
		Caco-2	ND	6.6 ± 0.4	ND	4.7 ± 0.3*
Ciprofloxacin	OCT1, BCRP	Ileum	2.6 ± 1.2	11.6 ± 3.1	3.1 ± 1.2	14.4 ± 2.3
Glipizide	Not known	Ileum	12.4 ± 1.1	19.5 ± 3.2	21.3 ± 5.2	22.1 ± 2.6
		Caco-2	4.7 ± 0.4	7.3 ± 0.9	7.5 ± 1.3*	8.3 ± 0.7
Atenolol	non	Ileum	4.4 ± 2.1	4.9 ± 1.5	4.9 ± 0.5	4.6 ± 0.4
Sildenafil	non	Ileum	11.9 ± 3.0	11.9 ± 4.2	11.6 ± 5.7	12.8 ± 0.7

* Statistically significant changes of P_{app} determined in the presence of AGE compared to the reference values of tested compound

ND the value of P_{app} in AP–BL direction for rosuvastatin could not be calculated, since the amount of compound permeating through Caco-2 cell monolayers was below the detection limit

verapamil, we concluded that the efflux of valsartan was mediated mainly by MRP-2. Based on that losartan was tested only in duodenum. In the case of pravastatin and atorvastatin Pgp and MRP-2 participated equally to their efflux.

The influence of 1 *v/v%* AGE on the permeability of other investigated compounds

The significantly higher S–M than M–S permeabilities for prazosin determined in the ileum with the highest BCRP and Pgp expression [28] confirmed that prazosin is actively secreted into the intestinal lumen by BCRP and/or Pgp. In the presence of AGE the efflux of prazosin increased, but the change was not significant ($\alpha = 0.061$).

For glipizide, an antidiabetic drug and according to our study (with Ver) not a Pgp substrate (data not presented), no changes in its permeability were observed after the addition of AGE either through the rat ileum or Caco-2 cell monolayers. The permeability of antibacterial compound ciprofloxacin, a substrate for basolateral efflux transporter OCT1 [26] was also not influenced by AGE. AGE had also no impact on the permeabilities of passively permeating drugs atenolol and sildenafil.

The permeability of rosuvastatin, a BCRP [23] substrate with even lower fraction of dose absorbed [29] than the other two statin representatives, was also evaluated. As shown in Table 6, AGE displayed different effects on BCRP transporters in the ileum than in Caco-2 cells. While it induced significant S–M permeability increase in the ileum, the BL–AP permeability through Caco-2 cells decreased significantly.

The influence of 1 *v/v%* mucosal/apical AGE on absorptive intestinal transporters

Prodrug valacyclovir imitates peptide structure and is actively absorbed with intestinal oligopeptide transporter PepT1 [30] located apically along the entire gastrointestinal tract [31]. Afterwards it is cleaved by enterocyte esterases to acyclovir, an antiviral drug [30]. For efficient di/tripeptide transport, PepT1 protein requires pH gradient. Therefore, to test AGE influence on PepT1 activity, pH on M/AP side of the jejunum and Caco-2 cells was set to 6.5, while S/BL pH was maintained at 7.4. AGE had opposite effects on valacyclovir M–S permeabilities through the rat jejunum and on the AP–BL permeability through the Caco-2 cell monolayers (Table 7). While the M–S permeability through the jejunum increased significantly in the presence of AGE, the AP–BL results obtained with Caco-2 cells indicated significantly lower permeabilities. In both experiments, 10 mmol/L amoxicillin was applied AP/M in the third phase of the experiment as a PepT1 inhibitor and the valacyclovir permeabilities decreased significantly only through the rat jejunum in its presence (Table 7).

The same pH-gradient conditions were also utilized for pravastatin and atorvastatin permeability determinations with AGE present in the M/AP solution. Both drugs are actively absorbed by OATP [30, 32] and MCT1 [33]. For pravastatin, permeability through Caco-2 cell monolayers could not be monitored, since HPLC analysis indicated profound metabolism of this statin in Caco-2 cells. According to the results in Table 7, the absorptive permeability of both statins increased significantly after the addition of AGE to the M/AP side. To ensure that the

Table 7 The influence of AGE on absorptive PepT1, MCT1 and OATP transporters through the rat jejunum and Caco-2 cell monolayers. The transporters responsible for drugs influx are also included in the table

Absorptive transporters	Transporter	P_{app} (*10^{-6} cm/s)				
			Ref	AGE	Inhibitor	
			M–S/AP–BL	M–S/AP–BL		M–S/AP–BL
Atorvastatin	OATP, MCT1	Jejunum	4.9 ± 1.2	10.0 ± 1.9*	CHC, DIDS	5.6 ± 2.6**
		Caco-2	1.8 ± 0.3	7.7 ± 2.1*	CHC, DIDS	1.9 ± 0.5**
Pravastatin	OATP, MCT1	Jejunum	6.3 ± 0.1	14.2 ± 3.5*	CHC, DIDS	10.1 ± 0.0**
Valacyclovir	Pept1	Jejunum	4.4 ± 1.0	7.1 ± 1.4*	Amox	1.0 ± 0.5**
		Caco-2	2.2 ± 0.2	1.7 ± 0.2*	Amox	1.5 ± 0.0

* Statistically significant changes of P_{app} determined in the presence of AGE or Inhibitors compared to reference values of tested compound

** Statistically significant changes of P_{app} determined in the presence of Inhibitor compared to P_{app} values in the presence of AGE

observed change was the consequence of MCT1/OATP increased activity, 1 mmol/L CHC (MCT1 inhibitor) and 0.5 mmol/L DIDS (OATP inhibitor) were used in the third phase of the experiments. Both inhibitors caused a significant decrease of pravastatin and atorvastatin permeabilities.

Discussion

In the first part of this study individual garlic phytochemicals were tested for their influence on Pgp. Hydrophilic compounds (SAC and low Q) increased Pgp efflux through both permeability models used, whereas the lipophilic ones did not affect the Pgp activity in Caco-2 cells but significantly increased Pgp efflux in ileal tissues. Because of Caco-2 cancerous origin, it is likely that Pgp overexpression in these cells represents a defensive mechanism towards cytostatic and other cytotoxic compounds (such as DADS [15] and MDS [4]). Therefore Pgp transporters are already maximally active enabling low intracellular concentration of these xenobiotic compounds and higher probability of cancerous cell survival. These noted discrepancies between the results obtained with lipophilic precursors might also result from differences in the cellular signaling pathways in normal and cancerous cells [13].

Increased Pgp mediated Rho123 efflux induced through the rat small intestine by AGE presence reported previously [13] is therefore most probably the result of an interplay of all investigated garlic ingredients. However, some authors [8] noticed moderate Pgp inhibition in the presence of garlic, which could be contributed to different garlic origin, processing methods, environmental conditions or different commercial product selected for the testing. The variability in all mentioned parameters could cause various Q (or other phytochemical) concentrations present in the final product and therefore different Pgp responses to different commercial garlic products. Because Pgp is also important in the liver drug transport, one could anticipate that Pgp modulating effects could occur in the liver also. After intestinal absorption, garlic phytochemicals present in AGE or other type of garlic supplements could increase the Pgp efflux of cytotoxic compounds from hepatocytes to bile, which would lead to lower intracellular drug/toxin concentrations resulting in higher hepatocyte vitality and furthermore to altered plasma drug/metabolite profile. Hepatoprotective effects of DADS and SAC were acknowledged by other authors [4], who noticed increased activity of phase II enzyme detoxifying enzymes (glutathione-*S*-transferase and glutathione peroxidase) in the presence of DADS and SAC.

Passive membrane permeability in the presence of AGE remained unaffected for tested drugs through the rat ileum (except for glipizide, glibenclamide and pravastatin), whereas permeabilities of all tested drugs significantly increased through Caco-2 cell monolayers. Because Caco-2 cells form a very tight monolayer (TEER values ranging from 600 to 800 Ωcm^2) compared to the rat small intestine (30–40 Ωcm^2) they can be considered as a highly sensitive model to investigate the role of various additives or solubilization agents as permeability enhancers. Minute alterations in membrane composition or tight junction opening could be noticed as a significant in vitro permeability change [34] similar to that observed in our study. However, because the reference average TEER values for Caco-2 cell monolayers did not differ significantly from those determined in the presence of AGE, the compounds' permeability changes were most probably caused by membrane fluidity or composition changes induced by AGE constituents and not by disrupting the tight junction architecture. The observed permeability changes in Caco-2 cells most probably do not reflect the real magnitude of the in vivo effect. In the case of glipizide, glibenclamide and pravastatin a significant M–S permeability increase through the

rat ileum induced by AGE could be the consequence of the same membrane fluidity and composition changes as observed with Caco-2 cells. Greater amounts of glipizide, glibenclamide or pravastatin absorbed could lead to higher plasma concentrations (perhaps above the expected maximal concentrations) and adverse drug reactions. Clinical studies showed that AGE consumption efficiently lowers plasma glucose levels and exerts cardioprotective effects [35], therefore a concomitant application of these three drugs and AGE could cause pharmacokinetic and pharmacodynamic interactions during conventional therapy.

The activity of efflux transporters Pgp and MRP-2 in the rat intestine and Caco-2 cell monolayers increased in the presence of AGE. The permeabilities in S–M direction of hydrochlorothiazide and digoxin, pure Pgp substrates tested, increased significantly in the presence of AGE and this could mean an insufficient absorption of both drugs, lower plasma concentrations and possibly lack of their pharmacological activity. Hydrochlorothiazide is a low permeable diuretic (Table 3), whereas digoxin is a drug with narrow therapeutic window applied in vivo in nanomolar concentrations. Glibenclanclamide BL–AP permeability through Caco-2 cell monolayers significantly decreased in contrast to the other pure Pgp substrates (digoxin and hydrochlorothiazide) indicating that the effect of AGE on intestinal transporters depends not only on the permeability model used and the level of transporter expression [13], but also on the tested compound itself.

The permeability of all tested mixed Pgp and MRP-2 substrates increased through Caco-2 cell monolayers, whereas significant efflux increase through the rat intestine was observed only for losartan, atorvastatin and pravastatin. Since all tested mixed substrates are drugs with low F_{abs}, low BU and/or low permeability (Table 3), MRP-2 and/or Pgp mediated liver transport and enzyme activities in the presence of AGE could also be significantly altered, therefore this aspect needs further evaluation. In the case of valsartan and losartan, extensive liver metabolism and enterohepatic cycling were reported to contribute to low BU (<40% BU [36, 37]), therefore inhibited intestinal absorption and lower F_{abs} together with lower hepatocyte metabolism in the presence of garlic supplements could lead to changes of plasma drug concentrations. For statins lower intracellular hepatocyte concentrations in the presence of AGE for both drugs could mean a decrease in their therapeutic potential, because liver is their main site of action. Furthermore AGE has been clinically proven (and is marketed for this indication) to alleviate symptoms of arteriosclerosis, for which statin therapy is usually prescribed, therefore these results definitely indicate a need for further investigation regarding pharmacokinetic and pharmacodynamic interactions [3, 4].

BCRP transporters expressed in rat ileum and Caco-2 cells responded differently to AGE addition. While the permeability of two substrates (prazosin, rosuvastatin) increased through the rat ileum, a decrease was observed in Caco-2 cells for rosuvastatin. Although Caco-2 cells are a good model to study interactions between different drugs regarding BCRP transporters [38], it might be possible that AGE phytochemicals interfere with intracellular signaling pathways involved in the BCRP regulation, which could differ between normal and malignant cells. Namely, in Caco-2 cells lower BCRP expression was observed [39], enabling malignant cells' higher profoporfirins retention. Therefore, the data obtained for rosuvastatin in ileum are most probably more physiologically relevant than the data from Caco-2 cell line. In the case of rosuvastatin, low BU [39] is mainly due to low permeability, since rosuvastatin metabolism is negligible (90% of dose in eliminated unchanged with bile and feces [40]). Additionally increased efflux of rosuvastatin with BCRP in the presence of AGE could lead to further decrease of its bioavailability and perhaps to an absence of the drug's therapeutic efficacy due to AGE influence on efflux transporters. However, when prazosin was utilized, the BCRP-mediated efflux increase was not significant, most probably because this is a Class I BCS compound, which can dissolve in gastrointestinal fluids to give sufficiently high final concentrations which saturate absorptive as well as secretory intestinal transporters. Therefore, the impact of intestinal secretory transporters on bioavailability of Class I compounds is clinically negligible despite concomitant consumption of transporter-activity modifying substances (food, supplements) as observed previously [7].

AGE phytochemicals also significantly increased the activity of absorbable MCT1, OATP and PepT1 transporters expressed in rat intestine for atorvastatin, pravastatin and valacyclovir, respectively. PepT1 transporters activity in Caco-2 cell decreased after addition of AGE, whereas MCT1 and OATP response was similar to that observed in intestine. Pharmacological interactions anticipated according to our results could probably also occur in vivo as altered fractions of the dose absorbed, especially for the drugs classified into II, III and IV BCS classes. The differences between PepT1 activity and regulation in the presence of AGE can be explained by the findings of Masereel et al. [41], who established that the homology of human multiple H^+-peptide transporters with the rat PepT1 is more than 80%. On the other hand, PepT1 cloned from Caco-2 cells have no homology and are unrelated to human hPepT1 [41]. Considering PepT1 homology differences between the rat small intestine and Caco-2 cells and the data of Anderle et al. [42], who noticed higher PepT1 activity in the presence of food flavonoids (also present in AGE), rat jejunum is most probably a better permeability

model to assess physiologically relevant AGE influence on PepT1 than Caco-2 cell monolayers.

References

1. Rivlin RS (2001) Historical perspective on the use of garlic. J Nutr 131(3S):951S–954S
2. Agarwal KC (1996) Therapeutic actions of garlic constituents. Med Res Rev 16:111–124
3. Ejaz S, Woong LC, Ejaz A (2003) Extract of garlic (*Allium Sativum*) in cancer chemoprevention. Exp Oncol 25:93–97
4. Yeh YY, Liu L (2001) Cholesterol-lowering effect of garlic extracts and organosulfur compounds: human and animal studies. J Nutr 131(3S):989S–993S
5. Amagase H (2006) Clarifying the real bioactive constituents of garlic. J Nutr 136:716S–725S
6. Fugh-Berman A (2000) Herb-drug interactions. Lancet 355:134–138
7. Wu CY, Benet LZ (2005) Predicting drug disposition via application of BCS: transport/absorption/elimination interplay and development of a biopharmaceutical drug disposition classification system. Pharm Res 22:11–23
8. Deferme S, Augustijns P (2003) The effect of food components on the absorption of Pgp substrates: a review. J Pharm Pharmacol 55:153–162
9. Foster B, Foster M, Vandenhoek S, Krantis A, Budzinski JW, Arnason JT, Gallicano KD, Choudri S (2001) An in vitro evaluation of human cytochrome P450 3A4 and P-glycoprotein inhibition by garlic. J Pharm Pharmaceut Sci 4:176–184
10. Lancaster JE, Boland MJ (1989) Flavor Biochem. In: Brewster JL, Rabinowitch HD (eds) Onions and allied crops: food science and minor crops. CRC Press, USA, pp 34–54
11. Jones MG, Hughes J, Tregova A, Milne J, Tomsett AB, Collin HA (2004) Biosynthesis of the flavour precursors of onion and garlic. J Exp Bot 55:1903–1918
12. Holub BJ, Arnott K, Davis JP, Nagpurkar A, Peschell J (2002) Organosulfur compounds from garlic. In: Shi J, Mazza G, Maguer M (eds) Functional foods, biochemical and processing aspects. CRC Press, USA, pp 214–231
13. Berginc K, Uršič D, Žakelj S, Kristl A (2009) Aged garlic extract stimulates P-glycoprotein and multidrug resistance associated protein 2 mediated effluxes. Biol Pharm Bull 32:694–699
14. Rosen RT, Hiserodt RD, Fukuda EK, Ruiz RJ, Zhou Z, Lech J, Rosen SL, Hartman TG (2001) Determination of allicin, S-allylcysteine and volatile metabolites of garlic in breath, plasma or simulated gastric fluids. J Nutr 131(3S):968S–971S
15. Amagase H, Petesch BL, Matsuura H, Kasuga S (2001) Intake of garlic and its bioactive components. J Nutr 131(S3):955S–962S
16. Shapiro AB, Ling V (1997) Positively cooperative sites for drug transport by P-glycoprotein with distinct drug specificities. Eur J Biochem 250:130–137
17. Yu DK (1999) The contribution of P-glycoprotein to pharmacokinetic drug-drug interactions. J Clin Pharmacol 39:1203–1211
18. Zhao YH, Abraham MH, Le J, Hersey A, Luscombe CN, Beck G, Sherborne B, Cooper I (2002) Rate-limiting steps of human oral absorption and QSAR studies. Pharm Res 19:1446–1457
19. Su SF, Huang JD (1996) Inhibition of intestinal digoxin absorption and exsorption by quinidine. Drug Metab Dispos 24:142–147
20. Soldner A, Benet LZ, Mutschler E, Christians U (2000) Active transport of the angiotensin-II antagonist losartan and its main metabolite EXP 3174 across MDCK-MDR1 and Caco-2 cell monolayers. Br J Pharmacol 129:1235–1243
21. Yamashiro W, Maeda K, Hirouchi M, Adachi Y, Hu Z, Sugiyama Y (2006) Involvement of transporters in the hepatic uptake and biliary excretion of valsartan, a selective antagonist of the angiotensin II AT1-receptors in humans. Drug Metab Dispos 34:1247–1254
22. Bakos E, Evers R, Sinko E, Varadi A, Borst P, Sarkadi B (2000) Interactions of the human multidrug resistance proteins MRP1 and MRP2 with organic anions. Mol Pharmacol 57:760–768
23. Wang E, Casciano CN, Clement RP, Johnson WW (2001) HMG-CoA reductase inhibitors (statins) characterized as direct inhibitors of P-glycoprotein. Pharm Res 18:800–806
24. Varma MVS, Sateesh K, Panchagnula R (2004) Functional role of P-glycoprotein in limiting intestinal absorption of drugs: contribution of passive permeability to P-glycoprotein mediated efflux transport. Mol Pharm 2:12–21
25. Zamek-Gliszczynski MJ, Hoffmaster KA, Humphreys JE, Tian X, Nezasa K, Brouwer KLR (2006) Differential involvement of Mrp2 (Abcc2) and Bcrp (Abcg2) in biliary excretion of 4-methylumbelliferyl glucuronide and sulphate in the rat. JPET 319:459–467
26. Žakelj S, Šturm K, Kristl A (2006) Ciprofloxacin permeability and its active secretion through rat small intestine in vitro. Int J Pharm 313:175–180
27. Guidance for industry bioavailability and bioequivalence studies for orally administered drug products — general considerations. Food and Drug Administration, Center for Drug evaluation and Research (CDER). Available via DIALOG http://www.fda.gov/cder/guidance/index.htm
28. Maclean C, Moenning U, Reichel A, Fricker G (2008) Closing the gaps–A full scan of the intestinal expression of Pgp, Bcrp and Mrp2 in male and female rats. Drug Metab Dispos 36:1249–1254
29. Lee E, Ryan S, Birmingam B, Zalikowski J, March R, Ambrose H, Moore R, Lee C, Chen Y (2005) Rosuvastatin pharmacokinetics and pharmacogenetics in white and Asian subjects residing in the same environment. Clin Pharmacol Ther 78:330–341
30. Steffansen B, Nielsen CU, Brodin B, Eriksson AH, Andersen R, Frokjaer S (2004) Intestinal solute carriers: an overview of trends and strategies for improving oral drug absorption. Eur J Pharm Sci 21:3–16
31. Faria TN, Timoszyk JK, Stouch TR, Vig BS, Landowski CP, Amidon GL, Weaver CD, Wall DA, Smith RL (2003) A novel high-throughput Pept1 transporter assay differentiates between substrates and antagonists. Mol Pharm 1:67–76
32. Kivistö KT, Niemi M (2007) Influence of drug transporter polimorphism on pravastatin pharmacokinetics in humans. Pharm Res 24:239–247
33. Katsura T, Inui KI (2003) Intestinal absorption of drugs mediated by drug transporters: mechanisms and regulation. Drug Metab Pharmacokinet 18:1–15
34. Sha X, Yan G, Wu Y, Li J, Fang X (2005) Effect of self-emulsifying drug delivery systems containing Labrasol on tight junctions in Caco-2 cells. Eur J Pharm Sci 24:477–486
35. Rahman K, Lowe GM (2006) Garlic and cardiovascular disease: a critical review. J Nutr 136(3):736S–740S
36. Flesch G, Muller PL (1997) Absolute bioavailability and pharmacokinetics of valsaratn, an angiotensin II receptor antagonist, in man. Eur J Clin Pharmacol 52:115–120
37. Lo MW, Goldberg MR, McCrea JB, Lu H, Furtek C, Bjornsson TD (1995) Pharmacokinetics of losartan, an angiotensin II receptor antagonist, and its active metabolite EXP3174 in humans. Clin Pharmacol Ther 58:641–649
38. Ebert B, Seidel A, Lampen A (2005) Identification of BCRP as transporter of benzo[a]pyrene conjugates metabolically formed in Caco-2 cells and its induction by Ah-receptor agonists. Carcinogenesis 26:1754–1763

39. Heimeur A, Conseil G, Deeley RG, Cole SPC (2004) The MRP-related and BCRP/ABCG2 multidrug resistance proteins: biology, substrate specificity and regulation. Current drug metabolism 5:21–53
40. Huang L, Wang Y, Grimm S (2006) ATP-dependent transport of rosuvastatin in membrane vesicles expressing breast cancer resistance protein. Drug Metab Dispos 34:738–743
41. Masereel B, Pochet L, Laeckmann D (2003) An overview of inhibitors of Na/H exchanger. Eur J Med Chem 38:547–554
42. Anderle P, Huang Y, Sadee W (2004) Intestinal membrane transport of drugs and nutrients: genomics of membrane transporters using expression microarrays. Eur J Pharm Sci 21:17–24
43. Gupta N, Martin PM, Miyauchi S, Ananth S, Herdman AV, Martindale RG, Podolsky R, Ganapathy V (2006) Down-regulation of BCRP/ABCG2 in colorectal and cervical cancer. BBRC 343:571–577
44. Shin HS, Bae SK, Lee MG (2006) Pharmacokinetics of sildenafil after intravenous and oral administration of rats: hepatic and intestinal first-pass effects. Int J Pharm 320:64–70
45. Halestrap AP, Meredith D (2004) The SLC16 gene family-from monocarboxylate transporters (MCTs) to aromatic amino acid transporters and beyond. Pflugers Arch 447(5):619–628
46. Gill RK, Saksena S, Alrefai WA, Sarwar Z, Goldstein JL, Carroll RE, Ramaswamy K, Dudeja PK (2005) Expression and membrane localization of MCT isoforms along the length of human intestine. Am J Physiol Cell Physiol 289:C846–C852