We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,300

Open access books available

172,000

International authors and editors

190M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

First-Pass Metabolism Changes After Long-Term Garlic Supplementation

Katja Berginc¹, Jurij Trontelj¹, Simon Žakelj¹,
Manica Černe² and Albin Kristl¹

¹*The Department of Biopharmacy and Pharmacokinetics, Faculty of Pharmacy, Ljubljana,*

²*Lek d.d., Ljubljana,
Slovenia*

1. Introduction

The suboptimal penetration of highly active antiretroviral therapy (HAART) drugs into the virus reservoirs and sanctuary sites ensures that the replication-competent HIV viruses or integrated HIV virions remain sheltered from HAART's pharmacological activity (Saksena et al, 2010). Persistent virus replication thus sustains continuous colonization of different tissues and cellular targets, which contributes to the disease relapse and to the emergence of virus resistance to HAART therapy (Saksena et al, 2010; Hoggs et al, 2006). Drug resistance is usually triggered by increased gene expression of the efflux transporters in the infected cells, resulting in even lower amounts of antiretrovirals residing in the HIV reservoirs (Löscher&Potschka, 2005; Kis et al, 2009). The persistent infection of the immune cells subjects HIV-infected to opportunistic infections (Orenstein et al, 1997), which alongside the metabolic syndrome, induced by long-term HAART therapy (Unger, 2003), contributes to the concomitant consumption of garlic supplements to ameliorate these symptoms (Amagase, 2006). However garlic phytochemicals have been shown to modulate the activities of intestinal (Berginc et al, 2009), and hepatic transporters (Berginc et al, 2010a) and CYP3A4 enzymes (Foster et al, 2001), the pivotal impediment to attaining therapeutic plasma concentrations after peroral administration and gastrointestinal absorption (Piscitelli et al, 2002; Vermier et al, 2009) of the protease inhibitors (i.e. saquinavir – Saq, darunavir – Dar) of human immunodeficiency virus (HIV-PI). Following that, concomitant application of antiretrovirals and garlic supplements could lead to pharmacokinetic interactions and possibly to therapy failure or to intensification of undesired effects. Piscitelli (Piscitelli et al, 2002) investigated the impact of long-term garlic supplements consumption (garlic caplets GarliPure® from Natrol) on the pharmacokinetics of Saq by conducting a clinical study on healthy male volunteers. A significant decline in the extent of saquinavir absorption was noted, which even after 10-day washout period did not return to the baseline values. The volunteers were administered higher Saq doses than needed for therapy (1200 mg 3-times daily); therefore the potential garlic modifying effects on the Saq pharmacokinetics could be even more pronounced among the infected patients, because they receive lower HIV-PI doses. Investigators suggested that the observed detrimental effect of garlic on the Saq pharmacokinetics could be caused by the formation of garlic metabolite(s) with enzyme-

transporter induction properties. Hypothetically, this would lead to the increased formation of Saq metabolites capable of inducing the metabolism of the parent drug. Plasma samples of the volunteers were not analyzed for the presence of potential garlic metabolites and the necessary *in vitro* studies to elucidate the hypothetical interaction mechanisms were never conducted. Therefore, these assumptions could not be verified. Furthermore, it has never been resolved, whether gut wall could represent an important hindrance to the absorption (Kis et al, 2009) and consequently to the therapeutic efficacy of HIV-PIs.

To address these questions, we performed *in vitro* studies with hepatic and intestinal tissues of rats, fed garlic supplements for two weeks. This approach allowed us to distinguish the influence, which garlic supplements could exert on first-pass Saq's metabolism in the gut and in the liver. Based on the knowledge of short-term influence of garlic phytochemicals and/or supplements on transporter-enzyme interplay (Berginc et al, 2009; Berginc et al, 2010a) and clinical data for Saq (Piscitelli et al, 2002), the mechanisms behind pharmacokinetic interactions between Saq and garlic were elucidated.

According to the clinical experience with Saq, a warning regarding the possibility of interactions with garlic or garlic supplements has been extended also to other HIV-PIs. Therefore, our research also aimed to identify, whether the "*in vitro*" transport and metabolism and perhaps therapeutic efficacy of Dar, a novel, second-generation HIV-PI, could really be affected by garlic consumption in a similar fashion. Furthermore, we hypothesized that differences may exist between the influences of different garlic supplements on HIV-PI pharmacokinetics. Rats were thus fed two garlic supplements with very distinct composition; aged garlic extract – AGE, a preferentially hydrophilic product rich in water-soluble γ -glutamyl derivatives of organosulfur compounds, flavonoids and other substances (Berginc et al, 2010b) and garlic oil macerate – GOM, containing preferentially oil-soluble allicin degradation products (i.e. various sulfides, ajoene, vinyl dithiins and other) (Yoshida et al, 1999). We also analyzed plasma samples of fed rats to identify potential garlic metabolites/phytochemicals, which could modify gene expression as suggested by Piscitelli (Piscitelli et al, 2002).

2. Methods and materials

2.1 Materials

Salts for incubation salines, sunflower seed oil from *Helianthus annuus*, Williams medium E, L-glutamine, insulin, gentamicin and ampicillin, analytical standards for constituents of garlic supplements (rutin, tangeretin, bergamottine, adenosine, S-allyl cysteine and quercetin) were from Sigma Aldrich Chemie (Deisenhofen, Germany). Saq and ritonavir (Rito) were purchased at Sequoia Research Products. All chemicals used in this study were of the highest grade available. Kyolic® liquid aged garlic extract (AGE) was produced by Wakanuga of America CO., LTD (Mission Viejo, CA, USA), lot number 5H04A. The AGE used in this study was standardized to 1.27 g/l S-allylcysteine content, the compound normally used for the standardization of AGE. Commercially available garlic's soft-gelatin capsules filled with garlic oil macerate (GOM) were purchased in a local pharmacy. Dar was extracted from Prezista® tablets (300mg) by ethanol extraction, evaporation and reconstitution in DMSO. The purity of extracted Dar was confirmed by HPLC analysis at 240 nm; the Dar peak surface represented 97% of the total chromatogram surface area.

2.2 Methods

2.2.1 Test system

The experiment was conducted with adult, at the start 12 weak old, male Wistar rats. The study conformed to the Law for the Protection of Animals (Republic of Slovenia) and was registered at the Veterinary Administration of Republic of Slovenia No. 34401-41/2008/2.

Animals were treated according to the principles of Convention ETS 123 (The convention for the protection of animals used for experimental and other scientific purposes) and Directive 86/609/EEC (Council directive on the protection of animals used for experimental and other scientific purposes).

Group	Supplemented with	N	Dosage	M _{before} (g)	M _{after} (g)
Sham-A group	saline	3	3.6 mL/kg	357	363
AGE group	AGE	3	1 % AGE in 3.6 ml of saline per kg	369	376
Sham-B group	sunflower seed oils	3	2.6 mL/kg	387	391
GOM group	GOM	3	0.3 % garlic oil macerate in 2.6 mL per kg	371	386

N – The number of fed rats; M_{before} - the mean rat weight before supplementation; M_{after} – the mean rat weight on day 15.

Table 1. The experimental design of chronic/long-term garlic supplementation to rats.

Animals were fed with pelletized feed Global Diet 2018 ad libidum and had free access to the drinking water through the experimental period. Animals were weighed before first application, on day 7 and at the end of the study. The first weighing was used for randomization, group formation and dose adjustments. From the first and last weighing of animals the mean body weight at the start and at the end of the study were calculated. The experiment was designed with 4 groups of animals. Two different garlic supplements (aged garlic extract – AGE or soft gelatin capsules with garlic oil macerates - GOM) were given orally to two groups daily by gavage for 14 days. The recommended human single oral doses of garlic supplements (2.5 mL of liquid AGE and 2 soft gelatin capsules of GOM, respectively) were adjusted according to the rat’s weight; AGE was diluted by saline and the content of soft gelatin capsules was diluted by sunflower seed oil (Table 1). Separately we also evaluated the impact of intragastric gavage of saline (sham-A group) and of sunflower seed oil (sham-B group), because they were used to dilute both garlic susppléments. The oil was chosen in compliance with OECD repeated dose methods where oil is recommended as vehicle for worse soluble items (OECD, 2008). Apart from intragastric gavage of different supplements/saline/sunflower seed oil, all groups had access to standard rat chow and water ad libidum. On day 15, rat small intestine, liver and plasma were obtained from rats after short fastening period before the sacrificing. After euthanasia and laparotomy, the intestine was rinsed with ice-cold 10 mM glucose Ringer solution and jejunum was used. Rat liver was preserved in ice-cold transportation medium, consisting of Williams medium E supplemented with L-glutamine (0.29

mg/mL), insulin (0.13 I.U./mL), gentamicin (50 µg/mL) and ampicillin (100 µg/mL). Plasma samples of rats fed with AGE, GOM and of reference animals were withdrawn and stored at -70°C until LC/MS/MS analysis.

2.2.2 Saq and Dar permeability through rat jejunum

The intestinal tissue was cut into 3 cm long segments, excluding visible Peyer's patches. Intestinal segments were opened along the mesenteric border, stretched onto inserts with exposed tissue area of 1 cm² and placed between two compartments of EasyMount side-by-side diffusion chambers (Physiologic Instruments, San Diego, USA). 2.5 mL of bathing solution (Ringer buffer, pH 7.4) on each side of intestine was maintained at 37°C and continuously oxygenated and circulated by bubbling with carbogen (95% O₂, 5% CO₂). 625 mM glucose and 625 mM mannitol were always added to the serosal (S) and mucosal (M) sides, respectively, to give 10 mM final concentrations. After 25 min of pre-incubation, incubation salines were replaced by donor solutions containing HIV-PIs at the highest concentrations, which could be prepared in Ringer buffer; Saq (25 µM) or Dar (100 µM). If monitoring mucosal-to-serosal (M-S) permeability, donor solution also contained 10 mM mannitol, whereas if monitoring serosal-to-mucosal (S-M) permeability, 10 mM glucose was present in the donor solution. 250 µL of samples were withdrawn from the acceptor (M or S) side every 20 min up to 80 min and replaced by the fresh Ringer buffer containing all necessary ingredients at appropriate concentrations. The donor solutions were also replaced before each sampling time to avoid the loss of investigated compound (Saq, Dar) due to its intestinal metabolism. Immediately after sampling, the withdrawn samples were precipitated by methanol (1:3), which contained haloperidol (100 µg/L) as internal standard, vortexed and kept on ice for at least 15 min. Afterwards the proteins were removed by centrifugation for 10 minutes at 15 000 g and 4°C. The supernatant was analyzed by LC/MS/MS for the parent drugs (Saq or Dar) and their metabolites.

During the permeability experiments, the diffusion chambers were equipped with two pairs of Ag/AgCl electrodes for measuring transepithelial potential difference and short circuit current with a multi channel voltage-current clamp (model VCC MC6, Physiologic Instruments) enabling the evaluation of tissue integrity and viability during the experiments. Only tissues that retained their integrity and viability (described in details in Berginc et al (Berginc et al, 2010b)) were used for the analysis.

During *in vitro* permeability experiments short-term and long-term effects were monitored. The expression short-term used throughout the text refers to the instant influence the addition of AGE supplement in 1 v/v % to the mucosal side of intestine from AGE or sham-A group exerted (i.e. allosteric modifications). On the other hand, the expression long-term effects refer to the transcriptional/translation changes resulting in different protein expression due to 2 week supplementation of rats.

2.2.3 Saq and Dar metabolism and transport in the rat liver slices

Rat liver slices (250 µm thick) for the experiments were prepared and pre-incubated for 30 min in the incubation medium (Williams medium E supplemented with L-glutamine (0.29 mg/mL), insulin (0.13 I.U./mL), gentamicin (50 µg/mL) and ampicillin (100 µg/mL)) at 37°C. Afterwards, each liver slice was incubated in 1.5 mL of the same medium, which also

contained 20 μ M Saq or Dar. The samples of incubation medium were withdrawn after 30 and 60 min for Saq and after 45 and 90 min for Dar to avoid steady state. After the incubation, liver slices were weighted and homogenized. All the samples were immediately precipitated with methanol (1:3) containing haloperidol as internal standard (100 μ g/L). The samples were afterwards prepared for the analysis in the same manner as described for permeability experiments. The LC/MS/MS analysis of the liver extract samples and of the withdrawn samples from the incubation medium at different time points was performed. The transport rate (the rate of metabolite concentration increase in the incubation medium) of two Saq and two Dar metabolites (Saq-M1, Saq-M3, Dar-M1, Dar-M4) was determined. In the liver extracts concentrations of Saq/Dar and their metabolites were measured. By comparing concentrations of the parent drugs and their metabolites in the intracellular cell compartment obtained by extraction and the metabolite transport rates with the corresponding reference values (i.e. concentrations of Saq/Dar and their metabolites or metabolite transport rates determined without garlic phytochemicals) we were able to determine the impact of garlic supplements on the activity of efflux/uptake transporters into bile or back into plasma) and CYP3A4.

2.2.4 Plasma preparation

Prior to the LC/MS/MS analysis, each 200 μ L plasma sample was precipitated with 3 volumes of ice-cold methanol, briefly vortexed, left to stand on -20°C for two days and then centrifuged at 10 000 g for 10 min at 4°C . The supernatant was transferred to autosampler vials for subsequent LC/MS/MS analysis.

2.2.5 LC/MS/MS sample analysis from studies with rat jejunum and liver slices

LC/MS/MS analysis of the samples obtained in the *in vitro* transport/metabolism experiments was performed on a Varian 1200L triple-quadrupole LC-MS Varian (Palo Alto, CA, USA). The mass spectrometer was coupled to ProStar 210 binary pumps, a Varian 420 autosampler and Varian 510 column oven. For chromatographic separation, a Phenomenex Kinetex 50x2.0 mm C-18 column with 2.6 μ m particles was used for Dar, and a Phenomenex Gemini 150x2.0 mm C-18 column with 3 μ m particles for Saq. The injection volume was 10 μ L and the column temperature was 50°C . Mobile phase consisted of water and acetonitrile containing 0.1% of formic acid. The elution for Dar and its metabolites was performed using a linear gradient from 20% to 65% of organic phase in 4 minutes with a flow rate of 0.4 mL/min, whereas that of Saq was performed using a linear gradient from 20% to 36% organic phase over 12 minutes with a flow rate of 0.5 mL/min. For the purpose of this study, two Dar (Dar-M1 – Dar carbamate hydrolysis (t_r 3.3 min), Dar-M4 – Dar hydroxylation (t_r 5.2 min)) [12] and two Saq (Saq-M1 – Saq oxidation at (1,1-dimethylethyl)amino group (t_r 8.1 min), Saq-M3 – Saq oxidation at benzyl group (t_r 5.9 min)) (Fitzsimmons&Collins, 1996) metabolites were monitored.

2.2.6 LC/MS/MS analysis of plasma samples

Rat plasma samples were analyzed by a triple-quadrupole LC/MS/MS Agilent 6460 (Agilent Technologies, Waldbronn, Germany) equipped with a Jet-Stream™ ESI interface coupled to an Agilent 1290 Infinity UPLC. 1 μ L of acetonitrile-precipitated rat plasma sample was injected on

a Phenomenex Synergi Polar-RP 100x3mm 2.5µm column. The mobile phase A consisted of water with 0.1% formic acid and mobile phase B consisted of 98% acetonitrile with 2% water. The gradient elution was linear from 1% B to 90% B over 10 minutes and the flow rate was 0.4 mL/min. After the column, the eluent was mixed with 0.2mL/min 100% acetonitrile via a zero-dead volume mixing tee in order to facilitate the evaporation and ionization process in the ESI Jet-Stream interface. The multiple reaction monitoring mode was used for the identification and relative quantification of AGE and GOM constituents in rat plasma (Table 4).

2.2.7 Data analysis

The apparent permeability coefficient (P_{app}) of HIV-PIs was calculated according to Eq. (1)

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

where dc/dt represents changes in HIV-PI concentration in the acceptor compartment per unit time under steady state conditions, V is the volume of the acceptor compartment, A is the exposed surface area (1 cm²) and c_0 is the initial, donor concentration of HIV-PIs. Afterwards the ratios (R_{ex}) were determined according to the Eq. (2) by dividing S-M P_{app} (P_{app}^{S-M}) values with the corresponding M-S P_{app} (P_{app}^{M-S}) values.

$$R_{ex} = \frac{P_{app}^{S-M}}{P_{app}^{M-S}} \quad (2)$$

Results in Tables and Figures are presented as means $\pm$ SD of at least 3 measurements. Data were evaluated statistically using SPSS 16.0 for Windows. Where appropriate, F-test for testing the equality of variances and, afterwards, 2-tailed Student t-test ($\alpha = 0.05$), were used. Otherwise, one way ANOVA, followed by Bonferroni post-hoc test were applied.

3. Results and discussion

3.1 The impact of long-term garlic supplementation on HIV-PIs intestinal permeability

The intestinal transporter-enzyme interplay (i.e. PGP and CYP3A4 or MRP-2 and UGT) can be responsible for lowering the fraction of the absorbed drugs that are subjected to the metabolism or transport with these proteins (Zhang et al, 2008). The PGP/MRP-2 mediated drug efflux from absorptive cells into the GIT lumen prolongs the overall residence time of drugs in the enterocytes through the more intense entero-enteric circulation, subjecting drugs to a more extensive intracellular phase I or phase II metabolism (Wu&Benet, 1999). The impact of long-term garlic supplementation on the activity of intestinal transporters and thus on the fraction of HIV-PIs (Saq, Dar) absorbed were assessed by evaluation of *in vitro* parent drug intestinal permeability through the rat jejunum (Table 2A, 2B). 100 µM Dar donor concentrations were chosen to simulate intraluminal Dar concentrations after peroral administration of clinical dose [12]. Since low water-solubility limits achieving high Saq concentrations (Aungst, 1999), the highest possible Saq donor concentrations (25 µM), which could be prepared in Ringer buffer pH 7.4, were applied.

Both HIV-PIs exerted low intestinal permeability regardless of whether rats were fed garlic supplements or not. Their M-S permeabilities were below 10×10^{-6} cm/s (Table 2A, 2B), the

limit above which drugs could be classified as highly permeable compounds according to our intra-laboratory experiments and FDA suggested highly permeable standards (Yu et al, 2002). Tested HIV-PIs were also profoundly effluxed from enterocytes into the lumen (high R_{ex} values, Table 2A and 2B - 1st phase designated “No Garlic”), indicating that at clinical Dar doses or the highest Saq doses, efflux transporters (Pgp, Mrp-2) were not saturated. This is in accordance with the results obtained by Holmstock (Holmstock et al, 2010). Namely, their Caco-2 permeability experiments and *in situ* intestinal perfusions of Pgp knock-down mice indicated a profound participation of Pgp in Dar’s secretion from absorptive cells even at the highest tested - clinical 100 μ M dose. Transporter saturation was also not achieved with 25 μ M Saq, as evident from significantly higher R_{ex} values (higher than 3, Table 2B) compared to the value 1, which usually indicates complete transporter inhibition. Since Pgp/Mrp-2 were not inhibited *in vitro*, the *in vivo* impact of efflux transporters on limiting drug absorption could also be significant, owing to the higher expression of transporters in human small intestine than in the rat’s (Cao et al, 2006). Therefore, gut represents an important biological barrier in achieving therapeutic plasma HIV-PI levels.

Table 2A						
Darunavir - P _{app} GIT (100 μ M) [cm/s]	No garlic			AGE addition		
	M-S (*10 ⁻⁷)	S-M (*10 ⁻⁶)	R _{ex}	M-S (*10 ⁻⁷)	S-M (*10 ⁻⁶)	R _{ex}
Sham-A group	7.7 ± 0.7	20.4 ± 1.0*	26	10.4 ± 4.4	20.6 ± 1.6*	20
AGE group	8.6 ± 0.5	13.4 ± 1.0 ^a	16	9.2 ± 1.1	11.8 ± 3.1*	13
Sham-B group	9.5 ± 2.1	17.9 ± 1.8*	19			
GOM group	8.1 ± 2.1	5.8 ± 1.7 ^a	7.2			

Table 2B						
Saquinavir - P _{app} GIT (25 μ M) [cm/s]	No garlic			AGE addition		
	M-S (*10 ⁻⁷)	S-M (*10 ⁻⁶)	R _{ex}	M-S (*10 ⁻⁷)	S-M (*10 ⁻⁶)	R _{ex}
Sham-A group	6.7 ± 0.3	2.6 ± 0.7*	3.9	5.9 ± 0.4	3.7 ± 0.3 ^b	6.3
AGE group	7.0 ± 2.0	5.6 ± 1.0 ^a	4	5.9 ± 0.5	7.4 ± 0.4 ^b	13
Sham-B group	6.9 ± 0.8	2.3 ± 0.2*	2.9			
GOM group	6.5 ± 2.7	4.9 ± 0.6 ^a	7.5			

HIV-PIs permeability was determined in two phases; first the permeability was measured in Ringer buffer pH 7.4 (20 min sampling intervals for 80 min – No garlic) and afterwards AGE was added to the mucosal side of rat ileum to give 1 % final AGE concentration and the experiment continued for additional 80 min (AGE addition).

* - S-M P_{app} HIV-PI values are significantly higher than the corresponding M-S P_{app} values
^a - S-M P_{app} values of treated rats (AGE or GOM group) significantly higher/lower than reference S-M P_{app} values (sham-A and sham-B group)
^b - S-M P_{app} values determined after the addition of AGE significantly changed compared to S-M P_{app} values in the first phase

Table 2. Saq and Dar intestinal permeability through rat jejunum determined in rats fed different garlic supplements (AGE or GOM – AGE and GOM group), saline (sham-A group) or sunflower seed oil (sham-B group). The permeability values of Saq and Dar after the addition of AGE are also given (AGE addition) in the table.

The short-term impact (i.e. instant/allosteric changes of transporter/enzyme activities) induced by AGE phytochemicals on intestinal rat's ABC transporters (Pgp, Mrp-2), involved in HIV-PI (Saq, Dar) absorption, have been investigated in our previous studies, where no feeding protocols were applied (Berginc et al, 2009; Berginc et al, 2010c). In the presented study these effects were re-investigated with the intestine of AGE fed rats to elucidate the (ir)reversibility of garlic phytochemicals or phytochemical metabolites binding to the efflux transporters. We reported previously that activities of Pgp and Mrp-2 significantly changed owing to allosteric modulations exerted in the presence of AGE components (Berginc et al, 2009; Berginc et al, 2010c). The impact of Pgp on the absorption of HIV-PIs was found to exceed that of Mrp-2, which has also been corroborated by Usansky (Usansky et al, 2008) in their *in vivo* study with Sprague-Dawley rats and Saq. In the case of Dar, mixed reports can be found regarding MRP-2/Mrp-2 importance in Dar absorption (Berginc et al, 2010c; Kakuda&Kiser, 2006). Based on this and the Mrp-2 expression pattern along GIT tract in rats (Mrp-2 expression decreases in more distal parts of rat's small intestine) (Berginc et al, 2010c; Usansky et al, 2008), the participation of Mrp-2 in HIV-PIs intestinal absorption is minor and the observed short-term effects in our studies were predominately caused by modified Pgp's activity. The noted short-term activity changes of the studied ABC transporters in our previous studies were explained by multiple binding sites in these transporters (four in Pgp and two in MRP-2) (Martin et al, 2000), which allow simultaneous binding of more substrates leading to allosteric modifications. Allosteric modifications induced by AGE phytochemicals in these studies were observed either as competitive inhibition between compounds (phytochemicals and drug) for an identical binding site (i.e. Dar – unchanged efflux) or as positive-cooperative effect and increased efflux (i.e. Saq), when the preferences of all involved substances for binding sites are distinct (Berginc et al, 2009; Berginc et al, 2010c). Similar allosteric modification of intestinal transporters involved in Saq and Dar efflux were also observed using small intestine of AGE fed rats in this study; Saq efflux significantly increased, whereas Dar efflux remained unchanged (slight but statistically insignificant decrease - Table 2A and 2B – phase "AGE addition"). The content of soft-gelatin capsules containing GOM could not be tested for their short-term influence on efflux transporters due to its poor miscibility with the incubation buffer, which would prevent adequate diffusion of oily phytochemicals from emulsion to the mucosal surface. The short-term modifications of efflux transporter activities were equally pronounced in rats, fed AGE, and in the control rats (sham-A group). Namely the average ratios between S-M P_{app} values after and before AGE addition were ca 1.0 for Dar and 1.5 for Saq, regardless which rats were used (treated or untreated – Table 2; sham-A and AGE group). This means that in the time-frame from the last intragastric gavage of garlic supplement and animal euthanization (18 h), garlic phytochemicals must have completely dissociated from intestinal efflux transporters to determine identical magnitude of permeability changes. Intestinal transporters in fed rats therefore could remain occupied with garlic phytochemicals after intragastric gavage but for at most 18 h per day. This could be sufficient to change transporter expression in the long run. However, transcriptional/translational effects and consequently elevated/decreased transporter expression could also be induced by binding of the absorbed xenobiotics on the transporter's gene promoters or to the corresponding transcriptional factors. Both scenarios could thus contribute to potential long-term expression changes. In the presented study mRNA or protein levels to quantify Pgp/Mrp-2

expression levels in (un)treated rats were not performed, because increased mRNA levels for Pgp have been found to correlate well with increased protein levels and its functionality (MacLean et al, 2008; Englund et al, 2006). Although such correlation between mRNA and protein levels for Mrp-2 has not yet been established (MacLean et al, 2008), diallyl disulfide, garlic sulfide present in oily (i.e. GOM) preparations and in smaller amounts also in aqueous/hydrophilic garlic supplements (i.e. AGE) (Berginc et al, 2009), induced transcription of Mrp-2, leading to increased Mrp-2 protein levels in rat renal brush-border membrane vesicles. Owing to the established positive correlation between increased Pgp/Mrp-2 levels (elevated protein and mRNA levels) and their functionality, the long-term impact of garlic constituents on the Saq and Dar intestinal permeability was assessed directly by measuring their permeabilities.

A 14-day AGE or GOM supplementation to rats significantly affected intestinal permeability of both antiretrovirals (Table 2A and 2B – AGE, GOM group); the efflux of Saq significantly increased and that of Dar significantly decreased compared to reference values (S-M P_{app} values of sham-A group – Table 2; see column “No garlic”). M-S permeabilities in both cases did not change (M-S P_{app} values sham-A or sham-B group). Sunflower seed oil, used to dilute GOM doses, had no impact on the permeability of tested HIV-PIs.

The increase of Saq's S-M P_{app} values observed in rats fed with both garlic supplements as mentioned, implies that the expression of efflux transporters in the mucosal enterocytic membrane must have increased during long-term garlic supplementation due to the repeated exposure of enterocytes to garlic phytochemicals or perhaps their metabolites. In fact, our results partially confirm a hypothesis which has been proposed by Piscitelli (Piscitelli et al, 2002) to explain lower amounts of absorbed Saq in a clinical study. Our results are in accordance with the part of hypothesis, which claims that the transporter expression changes could be induced by garlic phytochemicals or their metabolites. Since rats were not fed HIV-PIs and garlic supplements simultaneously as performed in clinical study by Piscitelli, the second part of his hypothesis that the parent drug (Saq) or its metabolites could also be responsible for the observed effects, could not be corroborated. However, in the case of Dar, in spite of the suggested increased transporter expression, the efflux of this HIV-PI was significantly lower than that determined for the untreated rats (sham-A group). As we mentioned previously, Dar and Saq Pgp binding sites are not identical, which was confirmed in previous *in vitro* study on rat intestine using ritonavir (Rito) as potent Pgp and Mrp-2 inhibitor (Berginc et al, 2010c). While Rito significantly inhibited Dar efflux from enterocytes regardless of the applied Rito concentration, that was not true for Saq. Instead of inhibiting Pgp-mediated Saq efflux, low Rito concentrations stimulated active Saq excretion and the inhibition was achieved only at saturable Rito concentrations. This undoubtedly indicated that Dar and Rito share identical binding site on Pgp, whereas Saq and Rito do not; only after Rito-Pgp binding site(s) are completely occupied by Rito, Rito can displace Saq from its binding site on Pgp. Therefore, significant decrease of Dar efflux after 2-week rat supplementation with garlic products observed in this study was most probably due to the occupation of Dar-binding site on Pgp by garlic phytochemicals or their metabolites with longer half-life(s). This indicates that the compounds from garlic supplements remained bound to intestinal transporters for at least 18h, additionally confirming Piscitelli's assumptions (Piscitelli et al, 2002).

3.2 The impact of long-term garlic supplementation on HIV-PIs hepatic pharmacokinetics

Various high-throughput screening studies using human hepatic microsomes (Foster et al, 2001) and recombinant CYP3A4 enzymes (Pal&Mitra, 2006) indicated that lipophilic garlic supplements, AGE and individual garlic phytochemicals could significantly inhibit CYP3A4 activity (short-term effect) and consequently impair HIV-PI (Saq, Dar) metabolism. The short-term effects of AGE on the hepatic HIV-PI distribution and metabolism in rat liver slices have also been investigated in our previous studies, where we have shown that AGE allosterically activated Pgp transport (i.e. it decreased intracellular concentrations of Pgp substrate - Dar) in the same manner as in the intestine but inhibited hepatic Mrp-2 transport. We determined that Mrp-2 inhibition observed in those studies was caused by metabolism of garlic flavonoids to the corresponding glucuronide metabolites, which are good MRP-2/Mrp-2 substrates. These glucuronide metabolites bind to Mrp-2 and competitively displace other Mrp-2 substrates (i.e. Saq) from these transporters, causing an increase of intrahepatic Saq concentrations. The inhibition of CYP3A4 HIV-PI metabolism by AGE was also confirmed in that study (Berginc et al, 2010d).

However after 14 days of AGE supplementation, the concentrations of both tested HIV-PIs in rat liver slices significantly increased (Table 3). Similar results were obtained also with rats fed with GOM, but the impact of GOM supplementation on the hepatic pharmacokinetics was obscured by the high influence of reference treatment - sunflower oil alone (sham-B group). Namely, if one compares intracellular concentrations of parent drugs and of the corresponding metabolites in the extracts from the liver slices of rats fed only sunflower oil (Table 3, sham-B group) with the corresponding values obtained from liver slices of rats fed saline (Table 3: sham-A group), it is obvious that sunflower oil itself exhibited pronounced effects on HIV-PI hepatic distribution and metabolism. The effect of GOM was thus more difficult to evaluate than that of AGE (Table 3; sham-A and AGE group). The daily quantities of the sunflower seed oil used to dilute GOM for sufficiently exact dosing and in the appropriate reference group was ca. 1 mL. According to the guidelines, this amount added to the rat's dietary fat intake does not influence the animal's condition regarding the clinical examination, hematological, biochemical and histological parameters (OECD, 2008). However, the increased daily fat intake was obviously sufficient to induce transcriptional/translational changes, which finally led to changes in HIV-PIs hepatic distribution and metabolism (significantly higher intracellular HIV-PI concentrations in rat liver slices and significantly higher formation and excretion rate of metabolites) compared to the reference values (Sham A group). Sunflower seed oil represents a good source of monounsaturated fatty acids (i.e. oleic acid), which have been known to reversibly increase the transcription of enzymes (among them also CYP) in liver and gut (Niot et al, 1997; Chen et al, 2001). The intake of higher fat quantities also interferes with endogenous lipid synthesis in metabolism, leading to changes in the plasma lipids and bile acid composition. To avoid cytotoxicity of the increased amounts of bile acids formed; these acids need to be excreted into the bile. Therefore, the increased production of bile acids had to be accompanied with the increased transporter expression to ensure cell survival. The excretion of bile acids into gallbladder involves the uptake of these endogenous compounds from plasma into hepatocytes with the aid of uptake transporters (i.e. NTCP, OATPs) and their excretion into the gallbladder by sinusoidal efflux proteins (Pgp, Mrp-2, S-Pgp, MDR3) (Meier et al, 1997a). Therefore the expression of these transporters in organisms

fed high fat diet would also increase, explaining the effects observed in this study on the hepatic HIV-PI pharmacokinetics in sunflower seed oil fed animals (sham-B group).

Table 3A	EXTRACT (mol/mg)			TRANSPORT RATE ($\mu\text{mol}/\text{min}\cdot\text{mg}$)	
	Saq ($\cdot 10^{-11}$)	Saq-M1 ($\cdot 10^{-13}$)	Saq-M3 ($\cdot 10^{-12}$)	Saq-M1 ($\cdot 10^{-9}$)	Saq-M3 ($\cdot 10^{-8}$)
Sham-A group	2.9 ± 0.3	4.6 ± 1.0	2.3 ± 0.1	3.8 ± 0.4	3.7 ± 0.3
AGE group	$8.2 \pm 0.2^*$	3.9 ± 0.7	2.4 ± 0.4	3.4 ± 0.3	3.2 ± 0.2
Sham-B group	$8.1 \pm 0.4^*$	4.9 ± 0.2	$4.7 \pm 0.7^*$	$5.6 \pm 0.6^*$	$6.4 \pm 0.6^*$
GOM group	$10.3 \pm 0.4^{*a}$	$7.8 \pm 0.7^*$	$4.3 \pm 0.9^*$	$4.8 \pm 0.1^*$	$6.0 \pm 0.3^*$

Table 3B	EXTRACT (mol/mg)			TRANSPORT RATE ($\mu\text{mol}/\text{min}\cdot\text{mg}$)	
	Dar ($\cdot 10^{-11}$)	Dar-M1 ($\cdot 10^{-11}$)	Dar-M4 ($\cdot 10^{-12}$)	Dar-M1 ($\cdot 10^{-8}$)	Dar-M4 ($\cdot 10^{-8}$)
Sham-A group	7.8 ± 0.6	2.5 ± 0.2	3.4 ± 0.2	3.6 ± 0.8	2.1 ± 0.2
AGE group	$10.3 \pm 1.0^*$	$1.7 \pm 0.0^*$	$1.7 \pm 0.6^*$	2.7 ± 0.5	1.8 ± 0.3
Sham-B group	$8.5 \pm 0.5^*$	2.8 ± 0.3	$6.0 \pm 0.4^*$	$6.1 \pm 1.4^*$	$5.9 \pm 0.1^*$
GOM group	$10.2 \pm 1.1^{*a}$	$3.2 \pm 0.2^*$	$5.0 \pm 0.7^*$	4.7 ± 0.5	$4.5 \pm 0.3^*$

* - the amounts of HIV-PIs and of the corresponding metabolites in the rat liver extracts or metabolite transport rates are significantly higher/lower than the corresponding reference values (sham-A and B group).

^a - significantly higher HIV-PI amounts in the extract compared to the amounts of HIV-PIs, determined in the extracts of rat liver extracts from control sham-A group.

Table 3. Saq (Table 3A) and Dar (Table 3B) hepatic *in vitro* pharmacokinetics evaluated in rat liver slices obtained from rats fed with different garlic supplements (AGE or GOM groups), saline (sham-A group) or sunflower seed oil (sham-B group). The amounts of HIV-PIs and of the corresponding metabolites in the rat liver extracts and the metabolite transport rates from the rat liver slices into the incubation medium were determined.

Higher intrahepatic retention of HIV-PIs after prolonged garlic consumption could thus be explained by two factors. First, hepatocytes were obviously exposed to lower concentration of xenobiotics or their metabolites, responsible for the induction of Pgp compared to enterocytes. Namely after peroral absorption of garlic phytochemicals, lower amounts of garlic phytochemicals and their corresponding metabolites could reach hepatocytes due to incomplete/low xenobiotic absorption through the intestinal mucosa, binding to enterocyte's mucosal membranes, instability in GIT lumen and intraluminal metabolism, which altogether contributed to less profound effect on the expression of efflux transporters in hepatocytes than in the jejunum (Ioannides, 2003; Lawson&Wang, 2005b). Besides efflux transporters, Saq and Dar also utilize OATP1A2 (OATP-A), 1B1 (OATP-C) and 1B3 (OATP-8) solute uptake transporters to distribute into the human liver (Hartkoorn et al, 2010). The sinusoidal rat hepatocyte membrane embodies numerous Oatp isoforms with Oatp1, Oatp2 and Oatp4 being the most important ones (Treiber et al, 2004; Meier et al, 1997b). Because AGE has been shown to affect Oatp activity (Berginc et al, 2010b), increased intrahepatic HIV-PI concentrations observed in this study could also correspond to the increased Oatp expression in the liver, which by its influence on the HIV-PIs pharmacokinetics could have exceeded the effects of efflux transporters.

These results suggest that the observed hepatic physiological adaptations to supplementation of garlic products or sunflower seed oil were exerted by garlic phytochemicals, components of sunflower seed oil and/or the corresponding metabolites. Because AGE lacks oily compounds that constitute GOM and/or sunflower seed oil, the substances responsible for the changes in membrane transporter expression, were most probably different. The differences in garlic supplement composition and/or plasma levels of garlic phytochemicals notwithstanding, the same final effect regarding HIV-PIs and HIV-PI-metabolite hepatic distribution were observed. The composition dissimilarities of garlic supplements and consequently of the produced metabolites also affected HIV-PIs Cyp3A metabolism in the liver slices differently. According to our results (Table 3), less Saq and especially Dar metabolites were formed in AGE group compared to the control sham-A group. However, in the presence of GOM and sunflower seed oil, Cyp metabolism of both investigated drugs significantly increased (note the significant increase of Saq-M1 and Dar-M4 in Table 3). Our results are in accordance with experimental data obtained in studies with various oily garlic supplements and AGE (Fischer et al, 2007). Garlic oil constituents, diallyl sulfide, diallyl disulfide and diallyl trisulfide have been found to increase the transcription of several hepatic enzymes (Cyp1A1, 2B1 and 3A1, NAD(P)H quinone oxidoreductase 1, glutathione-S-transferase) in wild type Wistar-Kyoto rats (Fischer et al, 2007), similarly to our findings in GOM group (increased production of HIV-PI metabolites and consequently their faster transport from hepatocytes into the incubation media). However, another clinical study on volunteers taking garlic extract (GarliPure®) twice daily for 3 weeks indicated no impact of garlic phytochemicals on CYP3A4 expression in intestine or liver, while at the same time PGP expression significantly increased (Hajdaa et al, 2010). GarliPure® extract is labeled to contain γ -glutamylcysteines (12 mg), alliin (4.8 mg), sulfur (3.9 mg), thiosulfinates (3.8 mg) and to have an allicin releasing potential, meaning that after consumption, this product enables *in vivo* allicin synthesis from alliin in ca 3.6 mg quantities. AGE, which we used to feed rats in this study, also predominately contains γ -glutamylcysteines with S-allyl-L-cysteine being the main representative. However, AGE does not contain allicin because during the aging process, the content of lipophilic allicin degradation products declines and the compounds responsible for distinct garlic odor and unwanted side effects in AGE are thus minimized. However, based on our analysis, small amounts of these lipophilic phytochemicals are still present in AGE (Berginc et al, 2009). A recent analysis by Lawson and Wang (Lawson&Wang, 2001a) indicated that only one of 24 marketed allicin-releasing brands enabled the release of the designated amount of allicin, whereas the release from other supplements reached only 10 to 15% of the declared values. Therefore, one can assume that the compositions of GarliPure® and of AGE used in this study are very similar. Similar to observations in the study with GarliPure® impact on Saq pharmacokinetics in humans (Hajdaa et al, 2010) we also determined no impact of long term AGE supplementation on Cyp3A Saq metabolism in rats, indicating that the expression of this enzyme remained constant in spite of AGE administration. However in the case of Dar, AGE significantly decreased its metabolism. Similarly to multiple binding sites in efflux transporters, the CYP3A4 kinetic studies by Shou (Shou et al, 1994) performed on CYP3A4 transfected HepG2 cells indicated the existence of two binding sites in this enzyme. It is thus possible, that Saq and Dar, although metabolized by the same enzyme (i.e. CYP3A4), do not share identical binding sites on CYP3A4, which would be similar to their interaction with Pgp transporters. Phytochemicals or metabolites present in or derived from AGE (but not GOM) could have occupied the Dar but not Saq binding site on CYP3A enzyme, similarly as

in the case of Pgp transporter. This resulted in altered Dar but not Saq metabolism. However this hypothesis will be difficult to confirm until more structural and functional information is available about the binding sites of CYP3A enzymes.

3.3 Plasma levels of AGE and GOM constituents and their metabolites

The plasma levels of AGE and GOM constituents in plasma samples taken 24 hours after the last dosing were very low, resulting in signals near the limit of detection on a top-of-the line LC/MS/MS system. This fact primarily confirms that we were really observing the long-term influences of AGE and GOM exerted through altered enzyme and transporter expression in our experiments with the tissue samples rather than short-term allosteric or competitive effects of garlic constituents remaining in the isolated animal tissues. Nevertheless, significantly higher levels of garlic constituents were obtained in the plasma from AGE group compared to the plasma of GOM group (Table 4). The levels of S-allyl-L-cysteine, tangeretin and quercetin were 2, 5.4 and 8 times higher, respectively, in the plasma of AGE group compared to the GOM group. The relatively long period between the last dose and the plasma sampling allowed the majority of garlic-related metabolites to be excreted, so the only metabolite that was confidently quantified, was bergamottine glucuronide and it was present in the AGE group. The much higher concentration of garlic constituents and metabolites in the plasma of AGE group is in accordance with the results from the rat liver slices experiment, where it was shown that the influence of AGE supplementation on the Saq and Dar liver disposition was beyond that of GOM's (Table 3). It could be argued that the majority of GOM constituents and their metabolites were already eliminated especially through the lungs (they are volatile) before we performed experiments with the liver slice and before the plasma samples were taken, since the rats were fasting for 18 hours before the sacrifice. On the other hand, the long term changes of hepatic protein expression caused by GOM supplementation should have remained relatively unchanged after 2 weeks in spite of short period of food and GOM deprivation. Therefore, the conclusion of AGE's greater influence on HIV-PI's hepatic disposition remains unchanged.

Analyte	MRM [m/z] (polarity)	ret. time [min]	Plasma- AGE [Area cps]	Plasma- GOM [Area cps]	Plasma- REF [Area cps]
Rutin	611->303 (+)	4,81	34	N/D	N/D
Bergammotine	339->203 (+)	10,35	N/D	N/D	N/D
S-allyl-L-cysteine	162->145 (+)	2,32	1174	593	152
Adenosine	268->136 (+)	2,62	3041	2079	515
Tangeretine	373->343 (+)	8,55	1227	229	306
Quercetine	301->151 (-)	6,37	412	51	116
Bergammotin-glucuronide	515->339 (+)	6,96	1088	N/D	N/D

N/D - the response was below the limit of detection

Table 4. The multiple reaction monitoring mode (MRM) transitions used for the LC/MS/MS identification and quantification of garlic constituents in rat plasma (first two columns) and the peak areas obtained from the plasma samples of rats fed with AGE, GOM and the reference group (the last three columns: Plasma-AGE, Plasma-GOM and Plasma-REF, respectively).

Furthermore, based on the plasma profiles from rats exposed to different garlic supplements, it can be concluded that constituents and their metabolites, although structurally and chemically different, induced similar hepatic effects regarding the transporter expression but exerted significantly different effect on CYP3A metabolism of both compounds. The affinities of these metabolites or their parent phytochemicals from both supplements for CYP3A are evidently different, because AGE constituents/metabolites inhibited/did not affect Cyp3A HIV-PI metabolism, whereas HIV-PI Cyp3A metabolism increased after long-term GOM supplementation.

4. Conclusion

Although applied at the highest possible donor concentrations, both drugs (Saq, Dar) displayed very low permeability in the absorptive direction and were subjected to a profound efflux from enterocytes back into intestinal lumen, indicating that the intestinal wall represents an important biological barrier for attaining therapeutic HIV-PIs plasma levels.

A concomitant consumption of garlic supplements together with HIV-PIs places the infected patients at higher risk for therapy failure, because the components of garlic supplements and/or their metabolites readily bind to efflux transporters. This binding changes the activities of efflux transporters by allosteric modifications in short-term and leads to an increased expression in the long run. Although two garlic supplements with different compositions were used, ABC transporter activity in the intestine increased similarly in both cases. Long-term garlic supplementation also affected hepatic metabolism and distribution of both HIV-PIs. Contrary to the effects in the intestine, the up-regulation of efflux transporters in the liver was less important than the increased expression of uptake Oatps transporters, which resulted in the overall increased intrahepatic HIV-PI amounts that could be further subjected to Cyp3A metabolism. Although the effects of AGE and GOM were similar regarding the transporter expression, their effects on Cyp3A metabolism were significantly different. AGE inhibited Dar but not Saq Cyp3A metabolism, while in GOM treated rats the Cyp3A metabolism of both drugs significantly increased, highlighting important dissimilarities in plasma profiles of garlic phytochemicals and the corresponding metabolites, as shown in this study. Given the fact that garlic supplements are so widely used in the HIV infected population because of their protective cardiovascular and anti-infective effects, further research should be directed at the identification of high- and low-risk supplement – drug combinations.

5. References

- Amagase, H. (2006) Clarifying the real bioactive constituents of garlic. *Journal of Nutrition*, Vol. 136, No. 3, pp. 716S – 725S, ISSN 0022-3166.
- Aungst, B.J. (1999). P-glycoprotein, secretory transport, and other barriers to the oral delivery of anti-HIV drugs. *Advanced Drug Delivery Reviews*, Vol. 39, No. 1-3 (October 1999), pp. 105 – 116, ISSN 0169-409X.
- Berginc, K., Žakelj, S., Uršič, D., Kristl, A. (2009). Aged garlic extract stimulates P-glycoprotein and Multidrug Resistance Associated Protein 2 mediated effluxes. *Biological&Pharmaceutical Buletin*, Vol.32, No. 4694, pp. 694 – 699, ISSN 0918-6158..

- Berginc, K., Trontelj, J., Kristl, A. (2010a) The influence of aged garlic extract on the uptake of saquinavir and darunavir into HepG2 cells and rat liver slices. *Drug Metabolism and Pharmacokinetics*, Vol. 25, No. 3, pp. 307 – 313, ISSN 1347-4367.
- Berginc, K., Žakelj, S., Kristl, A. (2010b). In vitro interactions between aged garlic extract and drugs used for the treatment of cardiovascular and diabetic patients. *European Journal of Nutrition*, Vol. 49, No.6, pp. 373 – 384, ISSN 1436-6207.
- Berginc, K., Trdan, T., Trontelj, J., Kristl, A. (2010c). HIV protease inhibitors: garlic supplements and first-pass intestinal metabolism impact on the therapeutic efficacy. *Biopharmaceutics & Drug disposition*, Vol. 31, No. 8-9, pp. 495 – 505, ISSN 0142-2782.
- Berginc, K., Milisav, I., Kristl, A. (2010d). Garlic flavonoids and organosulfur compounds: impact on the hepatic pharmacokinetics of saquinavir and darunavir. *Drug Metabolism and Pharmacokinetics*, Vol. 25, No. 6, pp. 521 – 531, ISSN 1347-4367.
- Cao, X., Gibbs, S.T., Fang, L., Miller, H.A., Landowski, C.P., Shin, H.C., Lennernas, H., Zhong, Y., Amidon, G.L., Yu, L.X.,
- Sun, D. (2006). Why is it Challenging to Predict Intestinal Drug Absorption and Oral Bioavailability in Human Using Rat Model. *Pharmaceutical Research*, Vol. 23, No. 8, pp. 1675 – 1686, ISSN 0724-8741.
- Chen, H.W., Yang, J.J., Tsai, C.W., Wu, J.J., Sheen, L.Y., Ou, C.C., Lii, C.K. (2001). Dietary fat and garlic oil independently regulate hepatic cytochrome P₄₅₀ 2B1 and the placental form of glutathione-S-transferase expression in rats. *Journal of Nutrition*, Vol. 131, No. 5, pp. 1438 – 1443 ISSN 0022-3166.
- Englund, G., Rorsman, F., Rönnblom, A., Karlbom, U., Lazorova, L., Gråsjö, J., Kindmark, A., Artursson, P. (2006). Regional levels of drug transporters along human intestinal tract: Co-expression of ABC and SLC transporters and comparison with Caco-2 cells. *European Journal of Pharmaceutical Sciences*, Vol 29, No. 3-4, pp. 269 – 277, ISSN 0928-0987.
- Fisher, C.D., Augustine, L.M., Maher, J.M., Nelson, D.M., Slitt, A.L., Klaassen, C.D., Lehman-McKeeman, L.D., Cherrington, N.J. (2007). Induction of drug-metabolizing enzymes by garlic and allyl sulfide compounds via activation of constitutive androstane receptor and nuclear factor E2-related factor 2. *Drug Metabolism & Disposition*, Vol. 35, No. 6 (June 2007), pp. 995 – 1000, ISSN 0090-9556.
- Fitzsimmons, M.E., Collins, J.M.. (1996). Selective biotransformation of the human immunodeficiency virus protease inhibitor saquinavir by human small-intestinal cytochrome P4503A4. *Drug Metabolism and Disposition*, Vol 25, No. 2, pp. 256 – 266, ISSN 0090-9556.
- Foster, B.C., Foster, M.S., Vandenhoeck, S., Krantis, A., Budzinski, J.W., Arnason, J.T., Gallicano, K.D., Choudri, S. An *in Vitro* Evaluation of Human Cytochrome P450 3A4 and P-glycoprotein Inhibition by Garlic. (2001). *Journal of Pharmacy and Pharmaceutical Sciences*, Vol. 4, No. 2, pp. 176 – 184, ISSN 1482-1826.
- Hajdaa, J., Rentsch, K.M., Gubler, C., Steinert, H., Stieger, B., Fattinger, K. (2010). Garlic extract induces gastrointestinal P-glycoprotein, but exhibits no effect on intestinal and hepatic CYP3A4 in humans. *European Journal of Pharmaceutical Sciences*, Vol. 41, No. 5 (December 2010), pp. 729 – 735, ISSN 0928-0987.
- Hartkoorn, R.C., Kwan, W.S., Shallcross, V., Chaikan, A., Liptrott, N., Egan, D., Sora, E.S., James, C.E., Gibbons, S., Bray, P.G.,

- Back, D.J., Khoo, S.H., Owen, A. (2010). HIV protease inhibitors are substrates for OATP1A2, OATP1B1 and OATP1B3 and lopinavir plasma concentrations are influenced by SLCO1B1 polymorphisms. *Pharmacogenetics & Genomics*, Vol. 2, No. 2 (February 2010), pp. 112 – 120, ISSN 1744-6872.
- Hogg, R.S., Bangsberg, D.R., Lima, V.D., Alexander, C., Wood, S.E., Dong, W.W.Y., Montaner, J.S.G., Harrigan, P.R. (2006). Emergence of drug resistance is associated with an increased risk of death among patients first starting HAART. *PloS Medicine*, Vol. 3, No. 9, (September 2006), pp. 1570 – 1578, ISSN 1549-1277.
- Holmstock, N., Mols, R., Annaert, P., Augustujns, P. (2010). In situ intestinal perfusion in knockout mice demonstrates inhibition of intestinal P-glycoprotein by ritonavir causing increased darunavir absorption. *Drug Metabolism and Disposition*, Vol. 38, No. 9 (September 2010), pp. 1407 – 1410, ISSN 0090-9556.
- Ioannides, C. (2003). Drug-phytochemical interactions. *Inflammopharmacology*, Vol. 11, No. 1, pp. 7 – 42.
- Kakuda, T.N., Kiser, J.J. (2006). 7th international workshop on clinical pharmacology of HIV therapy. *Expert Opinion on Pharmacotherapy*. Lisbon, Vol. 7, No. 11, pp. 1519 – 1533, ISSN 1472-4683.
- Kis, O., Robillard, K., Chan, G.N.Y., Bendayan, R. (2009). The complexities of antiretroviral drug-drug interactions: role of ABC and SLC transporters. *Trends in Pharmacological Sciences*, Vol. 31, No. 1 (January 2009), pp. 22 – 35, ISSN 0165-6147.
- Lawson, L.D., Wang, Z.J. (2001a). Low allicin release from garlic supplements: a major problem due to the sensitivities of allinase activity. *Journal of Agricultural and Food Chemistry*, Vol. 49, No. 5 (April 2001), pp. 2592 – 2599, ISSN 0021-8561.
- Lawson, L.D., Wang, Z.J. (2005b). Allicin and Allicin-Derived Garlic Compounds Increase Breath Acetone through Allyl Methyl Sulfide: Use in Measuring Allicin Bioavailability. *Journal of Agricultural and Food Chemistry*, Vol. 53, No. 6 (March 2006), pp. 1974 – 1983, ISSN 0021-8561.
- Löscher, W., Potschka, H. (2005). Drug resistance in brain diseases and the role of drug efflux transporters. *Nature Reviews Neuroscience*, Vol. 6, No. 8 (August 2005), pp. 591 – 602, ISSN 1471-0048
- MacLean, C., Moenning, U., Reichel, A., Fricker, G. (2008). Closing the gaps: A full scan of the intestinal expression P-glycoprotein, Breast Cancer Resistance Protein, and Multidrug Resistance Associated Protein 2 in male and female rats. *Drug Metabolism and Disposition*, Vol. 36, No. 7, (july 2008), pp. 1249 – 1254, ISSN 0090-9556.
- Martin, C., Berridge, G., Higgins, C.F., Mistry, P., Charlton, P., Callagan, R. (2000). Communication between Multiple Drug Binding Sites on P-glycoprotein. *Molecular Pharmacology*, Vol. 58, No. 3 (September 2000), pp. 624-632, ISSN 0026-895x.
- Meier, P.J., Eckhardt, U., Schroeder, A., Hagenbuch, B., Stieger, B. (1997). Substrate specificity of sinusoidal bile acid and organic anion uptake systems in rat and human liver. *Hepatology*, Vol. 26, No. 6 (December 1997), pp. 1667 – 1677, ISSN 0270-9139.
- Niot, I., Poirier, H., Besnard, P.H. (1997). Regulation of gene expression by fatty acids: special reference to fatty acid-binding protein (FABP). *Biochimie*, Vol. 97, No. 2-3 (February-March 1997), pp. 129 – 133, ISSN 0300-9084.

- OECD guidelines for the testing of chemicals 407 - Repeated Dose 28-Day Oral Toxicity Study in Rodents. 2008.
- Orenstein, J.M., Fox, C., Wahl, S.M. (1997). Macrophages as a Source of HIV During Opportunistic Infections. *Science*, Vol.7, No. 5320 (June 1997), pp. 1857 - 1861, ISSN 0036-8075.
- Pal, D., Mitra, A.K. (2006). MDR- and CYP3A4-mediated drug-herbal interactions. *Life Sciences*, Vol. 78, No. 18 (March 1997), pp. 2131 - 2145, ISSN 0024-3205.
- Piscitelli, S.C., Burstein, A.H., Welden, N., Gallicano, K.D., Falloon, J. (2002). The effect of garlic supplements on the pharmacokinetics of saquinavir. *Clinical Infectious Diseases*, Vol. 34, No. 2, pp. 234 - 238, ISSN 1058-4838.
- Saksena, N.K., Wang, B., Zhou, L., Soedjono, M., Ho, Y.S., Conceicao, V. (2010). HIV reservoirs in vivo and new strategies for possible eradication of HIV from the reservoir sites. *HIV/AIDS - Research and Palliative Care*, Vol. 2, pp. 103 - 122.
- Shou, M., Grogan, J., Mancewicz, J.A., Krausz, K.W., Gonzalez, F.J., Gelboin, H.V., Korzekwa, K.R. (1994). Activation of CYP3A4: Evidence for the simultaneous binding of two substrates in a Cytochrome P450 active site. *Biochemistry*, Vol. 33, No. 21 (May 1994), pp. 6450 - 6455, ISSN 0006-2960.
- Treiber, A., Schneider, R., Delahaye, S., Clozel, M. (2004). Inhibition of Organic Anion Transporting Polypeptide-Mediated Hepatic Uptake Is the Major Determinant in the Pharmacokinetic Interaction between Bosentan and Cyclosporin A in the Rat. *Journal of Pharmacology and Experimental Therapeutics*, Vol. 308, No. 3 (March 2004), pp. 1121 - 1129, ISSN 0022-3565.
- Unger, R.H. (2003). Lipid overload and overflow: metabolic trauma and the metabolic syndrome. *Trends in Endocrinology and Metabolism*, Vol. 14, No. 9 (November 2003), pp. 398 - 403, ISSN 1043-2760.
- Usansky, H.H., Hu, P., Sinko, P.J. (2008). Differential roles of P-glycoprotein, Multidrug-Resistance Associated Protein 2, and Cyp3A on Saquinavir oral absorption in Sprague-Dawley rats. *Drug Metabolism and Disposition*, Vol. 36, No. 5 (May 2008), pp. 863 - 869, ISSN 0090-9556.
- Vermeir, M., Lachau-Durand, S., Mannens, G., Cuyckens, F., van Hoof, B., Raoof, A. (2009). Absorption, metabolism, and excretion of darunavir, a new protease inhibitor, administered alone and with low-dose ritonavir in healthy subjects. *Drug Metabolism and Disposition*, Vol. 37, No. 4, pp. 809 - 820, ISSN 0090-9556.
- Wu, C.Y., Benet, L.Z. (2005). Predicting drug disposition via application of BCS: Transporter/absorption/elimination interplay and development of a biopharmaceutics drug disposition classification system. *Pharmaceutical Research*, Vol. 22, No. 1, pp. 11 - 23, ISSN 0724-8741.
- Yoshida, H., Katsuzaki, H., Ohta, R., Ishikawa, K., Fukuda, H., Fujino, T., Suzuki, A. (1999). An Organosulfur Compound Isolated from Oil-Macerated Garlic Extract, and Its Antimicrobial Effect. *Bioscience, Biotechnology and Biochemistry*, Vol. 63, No. 3, pp. 588 - 590, ISSN 0916-8451.
- Yu, L.X., Amidon, G.L., Polli, J.E., Zhao, H., Mehta, M.U., Conner, D.P., Shah, V.P., Lesko, L.J., Chen, M.L., Lee, V.H.L., Hussain, A.S. (2002). Biopharmaceutics Classification System: The Scientific Basis for Biowaiver Extensions. *Pharmaceutical Research*, Vol. 19, No. 7 (July 2002), pp. 921 - 925, ISSN 0724-8741.

Zhang, L., Zhang, Y.D., Strong, J.M., Reynolds, K.S., Huang, S.M. (2008). A regulatory viewpoint on transporter-based drug interactions. *Xenobiotica*, Vol. 38, No. 7-8, pp. 709 – 724, ISSN 0049-8254.

IntechOpen

IntechOpen

© 2011 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](https://creativecommons.org/licenses/by/3.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen