Quantification of CYP3A and drug transporters activity in healthy young, healthy elderly and chronic kidney disease elderly patients by a microdose cocktail approach

Punyabhorn Rattanacheeworn[1], Stephen J Kerr[2], Wonngarm Kittanamongkolchai[3], Natavudh Townamchai[4], Suwasin Udomkarnjananun[4], Kearkiat Praditpornsilpa[4,5], Thanundorn Thanusuwannasak[6], Udomsak Udomnilobol[7], Suree Jianmongkol[6,8], Boonsri Ongpipattanakul[6,9], Thomayant Prueksaritanont[7], Yingyos Avihingsanon[1,4], Pajaree Chariyavilaskul[1,10].

[1]Clinical Pharmacokinetics and Pharmacogenomics Research Unit, Chulalongkorn University, Bangkok, Thailand

[2]Biostatistics Excellence Center, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

[3]Maha Chakri Sirindhorn Clinical Research Center Under the Royal Patronage, Research Affairs, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

[4]Division of Nephrology, Department of Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

[5]Excellent Center of Geriatrics, King Chulalongkorn Memorial Hospital, Thai Red Cross Society, Bangkok, Thailand

[6]Chulalongkorn University Drug and Health Products Innovation Promotion Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand

[7]Chulalongkorn University Drug Discovery and Drug Development Research Center, Chulalongkorn University, Bangkok, Thailand

[8]Department of Pharmacology and Physiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand

[9]Department of Biochemistry and Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand

[10]Department of Pharmacology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

***Corresponding author:**

Pajaree Chariyavilaskul, M.D, Ph.D.

Department of Pharmacology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

Email: pajaree.l@chula.ac.th

Phone: +66816134664, +6622564481 ext 3020

Principal Investigator statement:

The authors confirm that the Principal Investigator for this paper is Punyabhorn Rattanacheeworn and that she had direct clinical responsibility for patients.

Clinical trial registration number:

TCTR 20180312002 registered on March 07, 2018.

Keywords:

Pharmacokinetics, Microdose cocktail, Cytochrome P450, Drug transporters, Elderly, Chronic kidney disease

Running title: Quantification of CYP3A and drug transporters activity in healthy young, healthy elderly and chronic kidney disease elderly patients by a microdose cocktail approach

Word counts: 3,397 (Introduction, Methods, Results, Discussion and Conclusion)

Tables: 5

Figures: 2

Pages: 24

What is already known about this subject

- Pharmacokinetics of special population changes due to the alteration of cytochrome P450 (CYP450) and drug transporters activity**.**
- Microdose cocktail developed for simultaneous determining CYP450 and drug transporters activity.
- No clinical pharmacokinetic studies have reported how do pharmacokinetic determinants exactly change in special population that could be important for drug uses.

What this study adds' statements

- This microdose cocktail clearly demonstrate a decreased in CYP3A activity which lead to increase 2-3 fold of plasma level in the elderly.
- The awareness of potentially adverse effects and drug-drug interactions should be concerned in the elderly.
- The trend of changes in drug transporter activity is also observed and yet to be concluded.

Abstract

Aims: Ageing and chronic kidney disease (CKD) are known to affect pharmacokinetics (PK) parameters. Since mechanisms are related and remain unclear, cytochrome P450 (CYP)3A and drug transporter activity were investigated in the elderly with or without CKD and compared to healthy adults using a microdose cocktail.

Methods: Healthy young volunteers (n = 20), healthy elderly volunteers (n = 16) and elderly with CKD (n = 17) received a single dose of microdose cocktail probe containing 30 μg midazolam, 750 μg dabigatran etexilate, 100 μg atorvastatin, 10 μg pitavastatin, and 50 μg rosuvastatin. After a 14-day washout period, healthy young volunteers continued to study period 2 with the microdose cocktail plus rifampicin. PK parameters including area under the concentration-time curve (AUC), maximum plasma drug concentration (C_{max}) and half-life were estimated before making pairwise comparisons of geometric mean ratios between groups.

Results: AUC and C_{max} of midazolam, a CYP3A probe substrate, were increased 2.30 and 2.90 fold in healthy elderly and elderly with CKD, respectively, leading to a prolonged half-life. AUC and C_{max} of atorvastatin, another CYP3A4 probe substrate, was increased 2.14 fold in healthy elderly and 4.15 fold in elderly with CKD, indicating decreased CYP3A4 activity related to ageing. Association with PK changes in probe drugs representing activity of OATP1B1, intestinal P-glycoprotein (P-gp), and breast cancer resistance protein (BCRP) transporters was noticed, but were inconclusive.

Conclusions: CYP3A activity is reduced in ageing. There is a trend in changes of OATP1B1, P-gp, and BCRP activity measured by microdose cocktail probe drugs.

1. Introduction

Chronic kidney disease (CKD) is a major public health problem worldwide, with impacts on national health expenditures and patient morbidity and mortality [1]. The overall prevalence of CKD in the Thai population is remarkably high (17.5% of the population), and the prevalence increases with advancing age [2]. Thailand is currently in a major demographic shift, with a rapid increase in the aging population and the elderly, defined in this study as those aged >60 years [3]. For renally excreted drugs, aging causes changes in drug pharmacokinetics, which are exacerbated in patients with CKD, leading to unexpected drug efficacy and safety [4]. Age-related alterations in liver function may also change drug metabolism and disposition through alteration of the cytochrome P450 (CYP) enzyme and drug transporter activity [5-8]. Because of multiple comorbid conditions, polypharmacy is also more common in aging patients. Physiologic changes in drug metabolism and clearance in aging patients increases the risk of adverse drug reactions and drug-drug interactions in this patient group [9, 10]

Clinical studies using probe substrate cocktails are recognized by both the United State Food and Drug Administration (U.S. FDA) and the European Medicines Agency (EMA) to evaluate the activity of multiple CYP enzymes and drug transporters simultaneously, together with their contribution to drug-drug interactions in man [11, 12]. Recently, a cocktail comprising a sub-therapeutic dose (micro-dose) of 5 drugs was validated in healthy subjects [13]. The cocktail drugs include 1) midazolam (MDZ), a specific and selective substrate for cytochrome P450 3A (CYP3A); 2) dabigatran etexilate (DABE), a selective substrate for gut P-glycoprotein (gut P-gp); 3) pitavastatin (PTV), a relatively selective substrate for organic anion-transporting polypeptide 1B; (OATP1B1); 4) atorvastatin (ATV), a substrate of cytochrome P450 3A4 (CYP3A4), P-gp, OATP, and breast cancer resistance protein (BCRP); and 5) rosuvastatin (RSV), a substrate of OATP and BCRP. This cocktail was subsequently applied to CKD patients [14] and the study results suggested that CKD reduces gut P-gp and BRCP activity [14]. However, whether the observed changes were due to CKD *per se,* or as a consequence of advancing age, remains inconclusive.

This study aimed to dissociate age- *versus* CKD-related changes, in the activity of CYP3A, and the drug transporters P-gp, OATP and BCRP, using a microdose cocktail in the elderly with and without CKD compared to young healthy volunteers.

2. Methods

This was a clinical pharmacokinetic study (Clinical trial registration number: TCTR 20180312002 registered on March 07, 2018). The study was approved by the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand and was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice. Written inform consent was obtained from all participants prior to the start of the study.

2.1 Participants

Participants were classified into 3 groups using pre-specified inclusion/exclusion criteria (Supplementary Table 1). Heathy young participants were recruited from the volunteers' database of Maha Chakri Sirindhorn Clinical Research Center under the Royal Patronage, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand. Healthy elderly participants were recruited from out-patients of the Comprehensive Geriatric Clinic, King Chulalongkorn Memorial Hospital, Thai Red Cross Society, Bangkok, Thailand. Elderly patients with CKD were recruited from an out-patient of the Nephrology Clinic, King Chulalongkorn Memorial Hospital, Thai Red Cross Society, Bangkok, Thailand by nephrologists in the study team.

2.2 Clinical study and biological sample collection procedures

All participants were advised to avoid xanthine containing beverages, citrus juice, herbal and dietary supplements, and products containing St. John's wort for 72 hours prior to study start, until study conclusion. Healthy young and healthy elderly volunteers abstained from any drug intake for at least 2 weeks prior to the study date. Elderly CKD patients continued their medications as usual, except for pitavastatin, atorvastatin, or rosuvastatin, which were stopped or switched to the equivalent dose of simvastatin at least 2 weeks prior to the study date.

The microdose cocktail containing 30 µg of MDZ, 750 µg of DABE, 10 µg of PTV, 100 µg of ATV, and 50 µg of RSV, was prepared by a single pharmacist trained in the study procedures, at the Pharmacy Department, King Chulalongkorn Memorial Hospital, Thai Red Cross Society, Bangkok, Thailand.

All participants received a single oral dose of the microdose cocktail in after 10 hours of overnight fast (study period 1). Venous blood samples were collected into an EDTA tubes at predose (0) and 0.33, 0.67, 1, 1.5, 2, 3,

4, 6, 8, 10, 12, 24, 36, and 48 hours post dose. Urine samples were collected at predose (0) and then between 0-4 hours, 4-8 hours, 8-12 hours, 12-24 hours, 24-36 hours, and 36-48 hours, post dose. Plasma and urine samples were aliquoted and kept at -80˚C until analysis. Samples for statins analysis were treated immediately with 1 M ammonium buffer (pH 5) at a ratio of 5/100 (buffer/plasma) to prevent interconversion. All participants stayed at the clinical study site for at least 12 hours post dose and returned for subsequent blood sample collections.

After a 2 weeks wash-out period, the young healthy volunteers continued to study period 2. On this occasion, participants received another single oral microdose cocktail together with 450 mg of rifampicin (RIF), a well-known OATP1B1, BCRP and gut P-gp inhibitor, to confirm the effect of microdose cocktail on drug transporters. Blood and urine samples on study period 2 were collected as previously described in study period 1. The observation for any adverse event was carefully monitored throughout the study.

2.3 Bioanalysis

Plasma concentrations of MDZ, DABI, PTV, pitavastatin lactone (PTV-lactone), ATV, 2-hydroxy atorvastatin (2-OH-ATV), 4-hydroxy atorvastatin (4-OH-ATV), RSV, RIF and isotope stable internal standard were quantified by a validated liquid chromatography tandem mass spectrometry assay as previously described [13]. Urine concentrations of DABI and RSV were also measured.

MDZ, statins and RIF were measured using reverse phase liquid chromatography. Hydrophilic interaction liquid chromatography with tandem mass spectrometric detection employing a turbo ion spray interface in positive ion mode was used for DABI. The lower limit of quantitation (LLOQ) was 1 pg/mL for MDZ, 20 pg/mL for DABI, and 5 pg/mL for statins and their metabolites.

2.4 Genotype analysis

The Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline recommends simvastatin drug monitoring and dose adjustment in patients based on *SLCO1B1* genotypic test results, as statins levels are affected by single nucleotide polymorphisms (SNPs) in drug transporters [15]. Therefore, we genotyped patients for solute carrier organic anion transporter 1B1 (*SLCO1B1)* and ATP Binding Cassette Subfamily G2 (*ABCG2*) genes, to adjust for this possible confounding factor [15-17]. The genotyping methods together with the genetic

variation in *SLCO1B1* c.521T>C (rs4149056), c.388A>G (rs2306283), g.-11187G>A (rs4149015), and *ABCG2* c.421C>A (rs2231142) distributions of all study participants have previously been reported [18].

2.5 Pharmacokinetic analysis

Pharmacokinetic data were analyzed using noncompartmental methods. The estimated pharmacokinetic parameters included maximum plasma concentration (C_{max}), time to maximum plasma concentration (T_{max}), area under the plasma concentration-time curve from time zero to the last measurable concentration ($AUC_{0\text{-}last}$) and AUC from time zero to infinity ($AUC_{0\text{-}inf}$), half-life ($T_{1/2}$) and renal clearance (Cl_R).

2.6 Statistical analysis

Statistical analysis was performed with STATA version 15.0 (Statacorp, College Station, TX, USA). Graphs were created by GraphPad Prism version 8.0 (GraphPad Software, Inc., San Diego, CA). Demographic data are presented as median (interquartile range; IQR) or frequency (%) for continuous and categorical data, respectively. Formal comparisons of continuous characteristics between groups were analyzed by a Kruskal-Wallis test, and when differences were found, pairwise comparisons were further investigated with a Wilcoxon rank-sum test. Categorical characteristics were compared with a Chi-square or Fisher's exact test as appropriate.

For pharmacokinetic parameters, the geometric mean (GM) with 95% confidence interval (95%CI) was calculated for $AUC_{0\text{-}last}$, $AUC_{0\text{-}inf}$, C_{max}, and Cl_R. Regression techniques with an outcome of the natural logarithm of each parameter were developed for these parameters, and pairwise comparisons were made between groups. The relevant model parameters were then exponentiated to obtain geometric mean ratios (GMR) and corresponding 95%CI. T_{max} and $T_{1/2}$ were reported as median (IQR) and pairwise comparisons made with a Kruskal-Wallis test/Wilcoxon test as for demographic parameters. Pre-RIF vs post-RIF comparison in healthy young volunteers was measured by a Generalized Estimating Equation (GEE). All p-values were adjusted for multiple comparisons using a Bonferroni correction. Multivariable models were used to adjust group differences for potential confounders including body mass index, alanine aminotransferase, aspartate aminotransferase, total bilirubin, direct bilirubin, albumin, genotype of *SLCO1B1* and *ABCG2* gene.

3. Results

3.1 Baseline characteristics

The total of 53 participants were enrolled. Twenty, 16 and 17 participants were healthy young adults, healthy elderly and elderly with CKD, respectively (Figure 1). As expected, 3 groups of participants were comparable except for age and renal function. The median age was 30, 65 and 74 years and the median eGFR was 112, 95 and 33 mL/min/1.73m^2 for healthy young volunteers, healthy elderly and elderly with CKD, respectively (Table 1).

3.2 Pharmacokinetic parameters of probe substrates

3.2.1 Midazolam

$AUC_{0\text{-last}}$, $AUC_{0\text{-inf}}$, C_{max} and $T_{1/2}$ of MDZ were significantly increased in healthy elderly volunteers and elderly CKD patients compared to healthy young adults (2.30 and 2.90 fold, respectively) but no significant differences seen between the groups of healthy elderly and elderly with CKD (Table 2, Figure 2A). RIF showed no effect on pharmacokinetics of MDZ (Table 2).

3.2.2 Dabigatran

$AUC_{0\text{-last}}$ and $AUC_{0\text{-inf}}$, but not C_{max} of DABI slightly increased with ageing (1.55 and 1.46 fold) while CKD patients showed a marked increase in drug exposure pharmacokinetic parameter ($AUC_{0\text{-last}}$ 4.11 fold, $AUC_{0\text{-inf}}$ 42.6 fold and C_{max} 1.70 fold) compared to healthy young controls. Elderly CKD patients had higher $AUC_{0\text{-last}}$ and $AUC_{0\text{-inf}}$ than healthy elderly with normal renal function (GMR (95% CI): 2.65 (1.91-3.69) and 2.91 (2.14-3.95), respectively) but no differences were observed in C_{max}. Of note, both healthy elderly and elderly with CKD had a reduction in DABI Cl_R (40% and 80% reduction, respectively) but only the CKD group had a very prolonged DABI $T_{1/2}$ (~ 3 fold, Table 2, Figure 2B). RIF significantly increased $AUC_{0\text{-last}}$, $AUC_{0\text{-inf}}$ and C_{max} of DABI and also reduced Cl_R of DABI by 25% (Table 2).

3.2.3 Pitavastatin and Pitavastatin lactone

Only elderly CKD patients showed a significant increase in $AUC_{0\text{-last}}$, $AUC_{0\text{-inf}}$, and C_{max} of PTV (1.67, 1.66 and 1.53 fold, respectively) and PTV-lactone (1.81, 1.87 and 1.35 fold, respectively) compared to healthy young adults. On the other hand, $AUC_{0\text{-last}}$ of PTV-lactone/PTV ratio were similar in all groups of participants (Table 3,

Figures 2C and 2D). RIF markedly increased $AUC_{0\text{-last}}$, $AUC_{0\text{-inf}}$ and C_{max} of PTV, but a lesser effect was observed with PTV-lactone, resulting in significant decreases in the PVT-lactone/PVT ratio (Table 3).

3.2.4 Atorvastatin and 4-hydroxy atorvastatin

$AUC_{0\text{-}last}$ and C_{max} of ATV and 4-OH-ATV were increased in healthy elderly (2.14 and 2.22 fold for ATV; 1.55 and 1.27 fold for 4-OH-ATV) and more prominently increased in CKD patients (4.15 and 4.18 fold for ATV; 2.58 and 1.84 fold for 4-OH-ATV) compared to healthy young participants (Table 4, Figures 2E and 2F). $AUC_{0\text{-}last}$ of 4-OH-ATV/ATV ratio was also reduced by approximately 20-30% compared to healthy adults (Table 4). RIF resulted in a highly increased $AUC_{0\text{-}last}$ and C_{max} of ATV and 4-OH-ATV, and with no effect on the 4-OH-ATV/ATV ratio (Table 4). Unfortunately, pharmacokinetic parameters of 2-OH-ATV could not be estimated as the plasma 2-OH-ATV concentrations were lower than the assay's LLOQ.

3.2.5 Rosuvastatin

Elderly CKD patients showed increased $AUC_{0\text{-}last}$ (1.90 fold) and C_{max} (1.63 fold) of RSV when compared to healthy young controls. Cl_R of RSV was considerably low in CKD patients (80% reduction) compared to 30% reduction in healthy elderly (Table 4, Figure 2G). $AUC_{0\text{-}last}$ and C_{max} of RSV were also increased with RIF (Table 4).

3.3 Multivariate analysis

Multivariate analyses were carried out for each drug in the microdose cocktail, adjusting for factors known to influence pharmacokinetic parameters. The results confirmed that all significant differences seen in the univariate analysis were independent from confounders (Tables 5).

3.4 Effects of genetic variations on pitavastatin, atorvastatin (*SLCO1B1*) and rosuvastatin (*ABCG2*) pharmacokinetics

The genetic variations of *SLCO1B1* gene did not affect pharmacokinetics of statins used in this study. In contrast, participants with *ABCG2* variants (421AA and 421CA genotypes, n = 24) showed increased $AUC_{0\text{-}t}$ and C_{max} of RSV compared to *ABCG2* wild type (Supplementary Table 2). When the participants with genotype variants (421AA and 421CA) were excluded from the multivariate analysis, the result showed that not only $AUC_{0\text{-}last}$ and C_{max} of RSV in the elderly CKD patients were higher than those of the healthy young volunteers as previously mentioned, but healthy elderly with normal renal function also had an increased $AUC_{0\text{-}last}$ (Supplementary Table 3 and Supplementary Figure 1).

3.5 Safety

Mild adverse events including diarrhea, dysmenorrhea, dyspepsia, migraine, and nasal congestion were observed in 6 participants. These adverse events were unrelated to the drug listed in the microdose cocktail. In the study period 2, healthy young volunteers had darkened urine as a result of RIF administration. Additionally, there were no significant changes in physical examination and clinical laboratories between baseline and at the end of study (Supplementary Table 4).

4. Discussion

Our study investigating the activity of CYP3A and drug transporters in 3 participant groups using a validated microdose cocktail containing 5 probe substrates, found CYP3A activity was reduced in aging. Changes in drug transporters activities appeared to be altered by either ageing or CKD.

Reductions in CYP3A activity were observed in both elderly groups, shown by the increased AUC and C_{max} and prolonged $T_{1/2}$ of MDZ, a CYP3A probe. Since MDZ is an intermediate hepatic extraction drug, a physiologically reduction of hepatic blood flow anticipated in the elderly might also contribute to the observed reduction in hepatic clearance of MDZ [19, 20]. CKD was not associated with changes in midazolam pharmacokinetics consistent with a previous study where neither AUC nor C_{max} of MDZ was affected by renal impairment [14].

In this study, AUC of DABI was increased in the elderly both with and without CKD compared to young adults. The magnitude of AUC augmentation was prominent in CKD patients and corresponded with a greater reduction in renal clearance in this group. However, the effect of gut P-gp activity as represented by DABI's pharmacokinetic changes in healthy elderly was still inconclusive. A study by Larsen, *et al*. reported no significant AUC elevation of digoxin, another probe substrate of P-gp at both the gut and systemic levels, in the elderly compared to young adults [21]. The association of CKD and gut P-gp activity was also studied in rats, and suggested a downregulation of gut P-gp activity in CKD, leading to increased drug absorption [22, 23]. The latest clinical study in CKD by Tatosian, *et al*. observed a progressive increase in DABI $AUC_{0\text{-}inf}$ and C_{max} with increasing severity of renal impairment up to 4.9 and 1.7 fold, respectively [14] which is similar to our data. Their finding was confirmed with the co-administration of RIF. Here, we also showed that the elderly without CKD also had a reduction of DABI clearance which may imply a reduction in gut P-gp activity. Although an effect of ageing or renal impairment on P-gp activity could not be concluded in this study, this finding is still of benefit to clinicians as most elderly are generally at risk of multiple drug treatments and drug-drug interactions.

PTV is a hepatic OATP1B1 substrate but the metabolite, PTV-lactone, is not. In this study, only OATP1B1 activity in elderly CKD patients was affected, demonstrated by increased AUC and C_{max} of PTV. It seems that ageing alone was not associated with a change in OATP1B1 activity. This is consistent with a previous pharmacokinetic study in patients with moderate and severe renal impairment without hemodialysis [24].

Moreover, reduced OATP1B1 transporter expression and activity were reported in a CKD rat model [25] and a physiologically-based pharmacokinetic study, where the reductions in hepatic OATP1B1 transporter activity of up to 60% were observed in patients with severe CKD [26]. Our findings were different from Tatosian, *et al*. [14] as we also observed an increase in PTV lactone, which is not an OATP1B1 substrate, and no difference in AUC ratio between PTV-lactone to PTV was observed in elderly CKD patients. Therefore, the effect of CKD on OATP1B1 transporter activity in our study is yet to be concluded. It is worth noting that our study and Tatosian, *et al*. measured total PTV level but also represented unbound PTV due to the same results of plasma protein binding test. Co-administration with RIF significantly increased AUC and C_{max} of PTV, but no effected on PTV-lactone and the AUC ratio between PTV-lactone to PTV was reduced. These findings supported the role of hepatic OATP1B1 influx transporter consistent with previous microdose cocktail study [13].

Consistent with other studies [17, 27, 28], we found that *ABCG2* polymorphisms showed effects on rosuvastatin, a substrate for BCRP and hepatic OATP1B1 transporter, evidenced by increased AUC and C_{max} in participants with *ABCG2* variant type. After excluding patients with this confounding genetic polymorphism in our multivariate analysis of RSV, we observed a significantly increased AUC in healthy elderly participants, and a more prominent increase in AUC in elderly CKD participants. This suggests that BCRP and hepatic OATP1B1 transporter activity might be affected by both ageing and renal impairment. However, a greater effect on C_{max} of RSV should have occurred if gut BCRP transporter activity was decreased. When considered with the results of PTV, a more selective substrate of OATP1B1, a definitive effect of renal impairment on hepatic OATP1B1 transporter activity is yet to be concluded. The association of ageing with BCRP and hepatic OATP1B1 transporter activity presented by changes with rosuvastatin level still cannot be fully determined in our study. Other studies reported no association between ageing and rosuvastatin exposure [29-31].

ATV as a probe substrate for CYP3A4, OATP1B1, BCRP, and P-gp, showed a significant increase in both AUC and $C_{max.}$ associated with ageing and renal impairment. ATV is converted by CYP3A4 into 4-OH-ATV as a major metabolite [32]. According to the decrease in the ratio of the AUC between 4-OH-ATV to ATV, the association of ageing and changes in ATV levels in this study could be related to a reduction in CYP3A4 activity which is comparable to those observed with MDZ. The effect of ageing on ATV pharmacokinetics suggestive of reduced CYP3A metabolizing enzyme activity was previously described [33]. The effects of renal dysfunction on ATV and its metabolite concentrations were still unable to be explained. However, augmentation

in AUC and C_{max} of ATV along with the severity of renal impairment together with a greater increment in C_{max} of ATV with RIF co-administration was stated [14]. Thus, BCRP and P-gp may also be additional factors involved in ATV pharmacokinetics alterations in CKD. Unfortunately, the BCRP and gut P-gp activity were inconclusive in this study due to the results of DABI and RSV. Efforts are now underway to develop a physiologically-based pharmacokinetic (PBPK) model using data from this study to help delineate the effect of aging and CKD on the underlying mechanisms related to these the drug transporters and drug metabolizing enzyme in elderly.

4.1 Study limitations

It has to be noted that during final part of the study period, MDZ tablet was not available in Thailand. Hence, intravenous form of MDZ was used in 4 elderly CKD patients instead and after dehydrating the solution was prepared to a power during the preparation for the microdose cocktail. Despite limited power, pharmacokinetic parameters between powder and solution of midazolam were similar and no significant difference was detected (Supplementary Table 5). RIF was co-administered only in young healthy participants in order to confirm the efficacy of the microdose cocktail.

5. Conclusion

The microdose cocktail approach provides a useful and safe screening tool to determine PK parameter alterations in populations with decreased CYP3A. Although changes in drug transporter activity could not be definitively concluded, our results suggest an age-associated trend in their changes. Health care providers should have a greater awareness of CYP3A drug substrates (such as midazolam, triazolam, simvastatin, ticagrelor, budesonide, sildenafil, felodipine, tacrolimus, etc) or CYP3A inhibitors (such as clarithromycin, erythromycin, ketoconazole, itraconazole, fluconazole, voriconazole, verapamil and several protease inhibitor antiretroviral drugs, etc) used in the elderly, since drug exposure to CYP3A drug substrates in the elderly could be increased 2-3 fold. Further studies with larger cohort sizes, that stratify based on CKD severity, together with patients on dialysis and RIF co-administration in all study populations is warranted. PBPK studies using data from this study would provide more information to inform predictions of changes in drug metabolizing enzymes and drug transporter activity in elderly with CKD.

Acknowledgements

We thank all participants who took part into this study. We also thank the clinical research staffs of Maha Chakri Sirindhorn Clinical Research Center Under the Royal Patronage, Research Affairs, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand for their clinical research support. Health Systems Research Institute, Ministry of Health, Thailand provided funding for this study. Rattanacheeworn, P. was supported by the 100th Anniversary Chulalongkorn University Fund for her training.

Conflict of interest

The authors report no conflict of interest to this work.

Funding information

Funding of this work was provided by Health Systems Research Institute, Ministry of Health, Thailand. Rattanacheeworn, P. was supported by the 100th Anniversary Chulalongkorn University Fund for Doctoral Scholarship.

Data availability

The data are available on reasonable request to the correspondence author.

Ethics approval

The study was approved by the Institutional Review Board (IRB) of the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand, and was conducted in compliance with the Declaration of Helsinki and the Principle of Good Clinical Practice (GCP).

Consent to participate

Written informed consents were obtained from all participants prior to the start of the study.

Consent to publish

All authors drafted the article and/or revised it critically for important intellectual content and provided final approval of the published version. Authors are responsible for correctness of the statement provided in the manuscript.

References

1. *World Population Ageing 2019*. affairs DoEaS; 2019.
2. Ingsathit A, Thakkinstian A, Chaiprasert A, et al. Prevalence and risk factors of chronic kidney disease in the Thai adult population: Thai SEEK study. *Nephrology Dialysis Transplantation*. 2009;25(5):1567-1575. doi:10.1093/ndt/gfp669
3. 10 Older population and health system: a profile of Thailand 2015 (2015).
4. Tieu A, House AA, Urquhart BL. Drug Disposition Issues in CKD: Implications for Drug Discovery and Regulatory Approval. *Advances in chronic kidney disease*. Mar 2016;23(2):63-6. doi:10.1053/j.ackd.2016.01.013
5. ElDesoky ES. Pharmacokinetic-pharmacodynamic crisis in the elderly. *American journal of therapeutics*. Sep-Oct 2007;14(5):488-98. doi:10.1097/01.mjt.0000183719.84390.4d
6. Klotz U. Pharmacokinetics and drug metabolism in the elderly. *Drug metabolism reviews*. 2009;41(2):67-76. doi:10.1080/03602530902722679
7. Yeung CK, Shen DD, Thummel KE, Himmelfarb J. Effects of chronic kidney disease and uremia on hepatic drug metabolism and transport. *Kidney international*. Mar 2014;85(3):522-8. doi:10.1038/ki.2013.399
8. Nolin TD, Naud J, Leblond FA, Pichette V. Emerging evidence of the impact of kidney disease on drug metabolism and transport. *Clinical pharmacology and therapeutics*. Jun 2008;83(6):898-903. doi:10.1038/clpt.2008.59
9. American Geriatrics Society 2019 Updated AGS Beers Criteria® for Potentially Inappropriate Medication Use in Older Adults. *Journal of the American Geriatrics Society*. Apr 2019;67(4):674-694. doi:10.1111/jgs.15767
10. Dagli RJ, Sharma A. Polypharmacy: a global risk factor for elderly people. *Journal of international oral health : JIOH*. Nov-Dec 2014;6(6):i-ii.
11. EMA. Guideline on the investigation of drug interactions. *Guid Doc*. 2012;44(June):59.
12. US Food and Drug Administration. Guidance for industry: drug interaction studies—study design, data analysis, implications for dosing, and labeling recommendations. *Center for Drug Evaluation and Research (CDER)*. 2012:1-75.
13. Prueksaritanont T, Tatosian DA, Chu X, et al. Validation of a microdose probe drug cocktail for clinical drug interaction assessments for drug transporters and CYP3A. *Clinical pharmacology and therapeutics*. Apr 2017;101(4):519-530. doi:10.1002/cpt.525
14. Tatosian DA, Yee KL, Zhang Z, et al. A Microdose Cocktail to Evaluate Drug Interactions in Patients with Renal Impairment. *Clin Pharmacol Ther*. Jul 23 2020;doi:10.1002/cpt.1998
15. Ramsey LB, Johnson SG, Caudle KE, et al. The clinical pharmacogenetics implementation consortium guideline for SLCO1B1 and simvastatin-induced myopathy: 2014 update. *Clinical pharmacology and therapeutics*. Oct 2014;96(4):423-8. doi:10.1038/clpt.2014.125
16. Romaine SP, Bailey KM, Hall AS, Balmforth AJ. The influence of SLCO1B1 (OATP1B1) gene polymorphisms on response to statin therapy. *The pharmacogenomics journal*. Feb 2010;10(1):1-11. doi:10.1038/tpj.2009.54
17. Keskitalo JE, Zolk O, Fromm MF, Kurkinen KJ, Neuvonen PJ, Niemi M. ABCG2 polymorphism markedly affects the pharmacokinetics of atorvastatin and rosuvastatin. *Clinical pharmacology and therapeutics*. Aug 2009;86(2):197-203. doi:10.1038/clpt.2009.79
18. Rattanacheeworn P, Chamnanphon M, Thongthip S, et al. SLCO1B1 and ABCG2 Gene Polymorphisms in a Thai Population. *Pharmgenomics Pers Med*. 2020;13:521-530. doi:10.2147/PGPM.S268457
19. Amrein R, Hetzel W. Pharmacology of Dormicum (midazolam) and Anexate (flumazenil). *Acta anaesthesiologica Scandinavica Supplementum*. 1990;92:6-15; discussion 47.
20. Dundee JW, Collier PS, Carlisle RJ, Harper KW. Prolonged midazolam elimination half-life. *Br J Clin Pharmacol*. Apr 1986;21(4):425-9. doi:10.1111/j.1365-2125.1986.tb05217.x
21. Larsen UL, Hyldahl Olesen L, Guldborg Nyvold C, et al. Human intestinal P-glycoprotein activity estimated by the model substrate digoxin. *Scand J Clin Lab Invest*. 2007;67(2):123-34. doi:10.1080/00365510600986084
22. Veau C, Leroy C, Banide H, et al. Effect of chronic renal failure on the expression and function of rat intestinal P-glycoprotein in drug excretion. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association*. Aug 2001;16(8):1607-14. doi:10.1093/ndt/16.8.1607
23. Naud J, Michaud J, Boisvert C, et al. Down-regulation of intestinal drug transporters in chronic renal failure in rats. *J Pharmacol Exp Ther*. Mar 2007;320(3):978-85. doi:10.1124/jpet.106.112631
24. Morgan RE, Campbell SE, Yu CY, Sponseller CA, Muster HA. Comparison of the safety, tolerability, and pharmacokinetic profile of a single oral dose of pitavastatin 4 mg in adult subjects with severe renal

impairment not on hemodialysis versus healthy adult subjects. *Journal of cardiovascular pharmacology*. Jul 2012;60(1):42-8. doi:10.1097/FJC.0b013e318256cdf0
25. Naud J, Michaud J, Leblond FA, Lefrancois S, Bonnardeaux A, Pichette V. Effects of chronic renal failure on liver drug transporters. *Drug Metab Dispos*. Jan 2008;36(1):124-8. doi:10.1124/dmd.107.018192
26. Tan ML, Zhao P, Zhang L, et al. Use of Physiologically Based Pharmacokinetic Modeling to Evaluate the Effect of Chronic Kidney Disease on the Disposition of Hepatic CYP2C8 and OATP1B Drug Substrates. *Clinical pharmacology and therapeutics*. Mar 2019;105(3):719-729. doi:10.1002/cpt.1205
27. Lee HK, Hu M, Lui S, Ho CS, Wong CK, Tomlinson B. Effects of polymorphisms in ABCG2, SLCO1B1, SLC10A1 and CYP2C9/19 on plasma concentrations of rosuvastatin and lipid response in Chinese patients. *Pharmacogenomics*. Aug 2013;14(11):1283-94. doi:10.2217/pgs.13.115
28. Zhang W, Yu BN, He YJ, et al. Role of BCRP 421C>A polymorphism on rosuvastatin pharmacokinetics in healthy Chinese males. *Clin Chim Acta*. Nov 2006;373(1-2):99-103. doi:10.1016/j.cca.2006.05.010
29. Kim Y, Yoon S, Choi Y, et al. Influence of OATP1B1 and BCRP polymorphisms on the pharmacokinetics and pharmacodynamics of rosuvastatin in elderly and young Korean subjects. *Sci Rep*. Dec 19 2019;9(1):19410. doi:10.1038/s41598-019-55562-4
30. Davidson MH. Rosuvastatin in elderly patients. *Drugs & aging*. 2007;24(11):933-44. doi:10.2165/00002512-200724110-00005
31. Martin PD, Dane AL, Nwose OM, Schneck DW, Warwick MJ. No effect of age or gender on the pharmacokinetics of rosuvastatin: a new HMG-CoA reductase inhibitor. *Journal of clinical pharmacology*. Oct 2002;42(10):1116-21. doi:10.1177/009127002401382722
32. Hoffart E, Ghebreghiorghis L, Nussler AK, et al. Effects of atorvastatin metabolites on induction of drug-metabolizing enzymes and membrane transporters through human pregnane X receptor. *Br J Pharmacol*. Mar 2012;165(5):1595-608. doi:10.1111/j.1476-5381.2011.01665.x
33. Gibson DM, Bron NJ, Richens A, Hounslow NJ, Sedman AJ, Whitfield LR. Effect of age and gender on pharmacokinetics of atorvastatin in humans. *J Clin Pharmacol*. Mar 1996;36(3):242-6. doi:10.1002/j.1552-4604.1996.tb04194.x

Table 1 Baseline characteristics.

Parameters	Healthy young adults	Healthy elderly	Healthy young adults *vs* healthy elderly	Elderly with CKD	Healthy young adults *vs* Elderly with CKD	Healthy elderly *vs* Elderly with CKD
n	20	16		17		
Male/ female (n/n)	8/12	4/12		13/4		
Age (year)	30 (28-32)	65 (62-67)	p <0.001	74 (67-77)	p <0.001	ns
Body mass index (kg/m^2)	22 (20-24)	23 (21-26)	ns	24.7 (23-27)	ns	ns
Biochemistry						
Blood urea nitrogen (mg/dL)	10 (10-12)	11 (10-14)	ns	26 (21-37)	p <0.001	p <0.001
Serum creatinine (g/dL)	0.7 (0.7-0.9)	0.7 (0.6-0.8)	ns	2.2 (1.8-2.9)	p <0.001	p <0.001
Estimated glomerular filtration rate (mL/min/1.73m^2)	112 (106-118)	95 (86-110)	ns	33 (24-40)	p <0.001	p <0.001
Parathyroid hormone (pg/mL)	55 (45-60)	63 (49-76)	ns	113 (76-168)	p <0.001	p <0.001
Fasting plasma glucose (mg/dL)	85 (81-93)	95 (90-102)	ns	98 (88-105)	ns	ns
Total protein (g/dL)	7.8 (7.5-7.9)	7.7 (7.3-7.8)	ns	7.6 (7.1-7.7)	ns	ns
Albumin (g/dL)	4.5 (4.3-4.6)	4.3 (4.3-4.5)	ns	4.3 (4.2-4.4)	ns	ns
Total bilirubin (mg/dL)	0.6 (0.5-0.8)	0.6 (0.6-0.9)	ns	0.6 (0.5-0.7)	ns	ns
Direct bilirubin (mg/dL)	0.2 (0.2-0.4)	0.3 (0.2-0.3)	ns	0.3 (0.2-0.3)	ns	ns
Aspartate aminotransferase (U/L)	16 (15-20)	22 (19-24)	ns	18 (16-26)	ns	ns
Alanine aminotransferase (U/L)	15 (13-20)	19 (16-22)	ns	19 (16-25)	ns	ns
Alkaline phosphatase (U/L)	54 (47-66)	67 (56-75)	ns	64 (53-73)	ns	ns
Total cholesterol (mg/dL)	203 (176-225)	242 (209-270)	ns	167 (152-177)	ns	ns
Triglyceride (mg/dL)	87 (61-107)	130 (94-191)	ns	91 (87-141)	ns	ns
Co-morbidity						
Hypertension	-	-		14 (82)		
Dyslipidemia	-	-		11 (65)		
Diabetes Mellitus	-	-		11 (65)		
Coronary heart disease	-	-		1 (6)		
Chronic kidney disease	-	-		17 (100)		
Osteoarthritis	-	2 (13)		1 (6)		
Benign prostatic hypertrophy	-	1 (6)		1 (6)		
Gout	-	1 (6)		1 (6)		

Data are presented in median (interquartile range) unless otherwise stated. CKD: chronic kidney disease; ns: non-significant.

Table 2 Pharmacokinetic parameters of midazolam and dabigatran.

Drugs	Healthy young adults			Healthy elderly		Elderly with chronic kidney disease	
	Microdose	Microdose + Rifampicin		Microdose		Microdose	
	GM (95% CI)	GM (95% CI)	GMR (95% CI)	GM (95% CI)	GMR (95% CI)	GM (95% CI)	GMR (95% CI)
Midazolam							
$AUC_{0\text{-last}}$ (pg/mL.hr)	192 (155-237)	248 (198-309)	1.29 (0.96-1.74)	440 (342-567)	2.30 (1.70-3.09)*	556 (454-681)	2.90 (2.16-3.88)*
$AUC_{0\text{-inf}}$ (pg/mL.hr)	200 (162-2 47)	256 (206-318)	1.28 (0.96-1.72)	464 (365-590)	2.32 (1.74-3.10)*	579 (475-705)	2.90 (2.18-3.85)*
C_{max} (pg/mL)	80 (67-96)	78 (96-118)	1.20 (0.92-1.56)	151 (119-191)	1.88 (1.44-2.44)*	156 (132-185)	1.95 (1.51-2.52)*
T_{max} (hr)[a]	0.7 (0.7-0.7)	0.7 (0.7-0.8)	-	0.7 (0.7-1.0)	-	0.7 (0.7-1.0)	-
$T_{1/2}$ (hr)[a]	2.7 (2.1-3.4)	2.5 (1.8-3.0)	-	5.9 (4.0-7.7)*	-	7.3 (5.3-8.1)*	-
Dabigatran							
$AUC_{0\text{-last}}$ (pg/mL.hr)	2,661 (2,147-3,298)	6,228 (5,165-7,511)	2.34 (1.78-3.08)*	4,118 (3,136-5,408)	1.55 (1.13-2.12)*	10,930 (8,714-13,708)	4.11 (3.01-5.61)*,¶
$AUC_{0\text{-inf}}$ (pg/mL.hr)	3,111 (2,577-3,757)	6,902 (5,849-8,146)	2.22 (1.74-2.83)*	4,557 (3,554-5,844)	1.46 (1.09-1.97)*	13,242 (10,480-16,732)	4.26 (3.18-5.70)*,¶
C_{max} (pg/mL)	375 (302-467)	699 (593-824)	1.86 (1.43-2.43)*	477 (378-601)	1.27 (0.94-1.73)	640 (506-808)	1.70 (1.26-2.30)*
T_{max} (hr)[a]	1.5 (1.0-1.5)	2.0 (2.0-2.5)*	-	1.5 (1.0-1.5)	-	1.5 (1.0-2.0)	-
$T_{1/2}$ (hr)[a]	6.6 (4.6-7.8)	7.4 (5.8-10.6)	-	6.9 (5.9-8.2)	-	16.8 (14.5-21.7)*,¶	-
CL_R, mL/min	78 (70-88)	59 (53-66)	0.76 (0.65-0.88)*	48 (39-59)	0.61 (0.48-0.78)*	15 (12-18)	0.19 (0.15-0.24)*,¶

[a]Data are presented in median (interquartile range).

*p-value<0.05, healthy young adult as a reference group.

¶p-value<0.05, healthy elderly as a reference group.

$AUC_{0\text{-inf}}$: area under the concentration-time curve of time zero to infinity; $AUC_{0\text{-last}}$: area under the concentration-time curve of time zero to the last time point; CI: confidence interval; CL_R: renal clearance; C_{max}: maximum plasma concentration; GM: geometric mean; GMR: geometric mean ratio; T_{max}: time to maximum plasma concentration; $T_{1/2}$: half-life.

Table 3 Pharmacokinetic parameters of pitavastatin and pitavastatin lactone.

Drugs	Healthy young adults			Healthy elderly		Elderly with chronic kidney disease	
	Microdose	Microdose + Rifampicin		Microdose		Microdose	
	GM (95% CI)	GM (95% CI)	GMR (95% CI)	GM (95% CI)	GMR (95% CI)	GM (95% CI)	GMR (95% CI)
Pitavastatin							
$AUC_{0\text{-}last}$ (pg/mL.hr)	429 (375-491)	1,956 (1,656-2,312)	4.56 (3.71-5.61)*	446 (375-530)	1.04 (0.82-1.31)	715 (578-885)	1.67 (1.33-2.09)*,¶
$AUC_{0\text{-}inf}$ (pg/mL.hr)	468 (405-540)	1,980 (1,677-2,339)	4.24 (3.42-5.24)*	494 (417-585)	1.06 (0.83-1.34)	774 (624-959)	1.66 (1.32-2.08)*,¶
C_{max} (pg/mL)	159 (132-191)	857 (729-1,008)	5.40 (4.25-6.86)*	154 (123-192)	0.97 (0.73-1.28)	242 (193-303)	1.53 (1.16-2.00)*,¶
T_{max} (hr)[a]	0.7 (0.7-0.7)	0.7 (0.7-0.7)	-	0.7 (0.7-0.7)	-	0.7 (0.7-0.7)	-
$T_{1/2}$ (hr)[a]	12.2 (10.7-14.1)	6.5 (4.8-9.6)*	-	12.6 (11.4-15.9)	-	15.0 (11.3-17.6)	-
Pitavastatin lactone							
$AUC_{0\text{-}last}$ (pg/mL.hr)	524 (440-623)	649 (556-759)	1.24 (0.99-1.56)	604 (494-739)	1.15 (0.89-1.50)	950 (767-1177)	1.81 (1.41-2.34)*,¶
$AUC_{0\text{-}inf}$ (pg/mL.hr)	574 (475-693)	690 (589-809)	1.20 (0.95-1.53)	676 (558-819)	1.18 (0.90-1.54)	1,071 (861-1,332)	1.87 (1.44-2.42)*,¶
C_{max} (pg/mL)	59 (51-68)	76 (67-86)	1.29 (1.08-1.55)*	48 (58-69)	0.98 (0.78-1.22)	80 (66-97)	1.35 (1.09-1.68)*,¶
T_{max} (hr)[a]	1.5 (1.0-1.8)	1.5 (1.0-2.0)	-	1.5 (1.5-1.5)	-	1.5 (1.5-2.0)	-
$T_{1/2}$ (hr)[a]	13.1 (8.6-17.6)	12.8 (8.9-18.1)	-	13.7 (11.6-17.9)	-	15.3 (12.2-19.0)	-
Pitavastatin lactone/Pitavastatin ratio							
$AUC_{0\text{-}last}$	1.22 (1.08-1.38)	0.33 (0.30-0.37)	0.40 (0.34-0.47)*	1.35 (1.22-1.51)	1.13 (0.89-1.43)	1.33 (1.15-1.54)	1.13 (0.90-1.41)

[a]Data are presented in median (interquartile range).

*p-value<0.05, healthy young adult as a reference group.

¶p-value<0.05, healthy elderly as a reference group.

$AUC_{0\text{-}inf}$: area under the concentration-time curve of time zero to infinity; $AUC_{0\text{-}last}$: area under the concentration-time curve of time zero to the last time point; CI: confidence interval; C_{max}: maximum plasma concentration; GM: geometric mean; GMR: geometric mean ratio; T_{max}: time to maximum plasma concentration; $T_{1/2}$: half-life.

Table 4 Pharmacokinetic parameters of atorvastatin, 4-hydroxy atorvastatin and rosuvastatin.

Drugs	Healthy young adults			Healthy elderly		Elderly with chronic kidney disease	
	Microdose	Microdose + Rifampicin		Microdose		Microdose	
	GM (95% CI)	GM (95% CI)	GMR (95% CI)	GM (95% CI)	GMR (95% CI)	GM (95% CI)	GMR (95% CI)
Atorvastatin							
$AUC_{0\text{-last}}$ (pg/mL.hr)	131 (102-168)	767 (609-965)	5.84 (4.21-8.10)*	281 (228-347)	2.14 (1.52-3.02)*	545 (411-725)	4.15 (2.98-5.79)*,¶
C_{max} (pg/mL)	16 (12-21)	258 (196-339)	16.37 (11.25-23.81)*	35 (27-45)	2.22 (1.49-3.31)*	66 (47-92)	4.18 (2.85-6.14)*,¶
T_{max} (hr)[a]	0.3 (0.3-0.3)	0.7 (0.7-1.0)*	-	0.3 (0.3-0.3)	-	0.3 (0.3-0.3)	-
4-Hydroxy atorvastatin							
$AUC_{0\text{-last}}$ (pg/mL.hr)	94 (74-120)	729 (590-901)	7.75 (5.67-10.58)*	146 (113-187)	1.55 (1.12-2.14)*	243 (194-304)	2.58 (1.88-3.53)*,¶
C_{max} (pg/mL)	7 (6-8)	109 (89-134)	16.08 (12.40-20.86)*	9 (7-11)	1.27 (0.94-1.72)	12 (9-17)	1.84 (1.37-2.47)*,¶
T_{max} (hr)[a]	5.0 (4.0-8.0)	2.0 (1.0-2.5)*	-	8.0 (6.0-10.0)	-	6.0 (4.0-10.0)	-
4-Hydroxy atorvastatin/Atorvastatin ratio							
$AUC_{0\text{-last}}$	0.72 (0.57-0.90)	0.95 (0.84-1.07)	1.20 (0.96-1.50)	0.52 (0.42-0.64)	0.78 (0.64-0.96)*	0.45 (0.37-0.53)	0.72 (0.59-0.87)*
Rosuvastatin							
$AUC_{0\text{-last}}$ (pg/mL.hr)	195 (133-288)	851 (690-1,050)	4.35 (2.84-6.67)*	290 (228-369)	1.48 (0.95-2.32)*	372 (279-495)	1.90 (1.23-2.93)*
C_{max} (pg/mL)	32 (23-45)	232 (177-305)	7.21 (4.78-10.89)*	37 (31-45)	1.15 (0.78-1.69)	53 (40-70)	1.63 (1.12-2.37)*
T_{max} (hr)[a]	3.5 (1.8-4.0)	1.0 (1.0-1.5)*	-	4.0 (3.0-4.0)	-	2.0 (1.0-3.0)	-
CL_R (mL/min)	223 (192-259)	147 (131-164)	0.66 (0.55-0.79)*	157 (126-195)	0.70 (0.51-0.97)*	42 (30-60)	0.19 (0.14-0.26)*,¶

[a]Data are presented in median (interquartile range).

*p-value<0.05, healthy young adult as a reference group.

¶p-value<0.05, healthy elderly as a reference group.

$AUC_{0\text{-last}}$: area under the concentration-time curve of time zero to the last time point; CI: confidence interval; CL_R: renal clearance; C_{max}: maximum plasma concentration; GM: geometric mean; GMR: geometric mean ratio; T_{max}: time to maximum plasma concentration; $T_{1/2}$: half-life.

Table 5 Multivariate analysis of the differences in $AUC_{0\text{-last}}$, C_{max} and half-life between 3 groups of participants.

Multivariate model	Midazolam	Dabigatran	Pitavastatin[a]	Pitavastatin lactone[a]	Atorvastatin[a]	4-Hydroxy atorvastatin[a]	Rosuvastatin[b]
n	53	53	52	52	52	52	29
$AUC_{0\text{-last}}$ (pg/mL.hr)							
Healthy young adults	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Healthy elderly	2.19 (1.61-2.98)**	1.65 (1.15-2.35)*	0.99 (0.79-1.25)	1.14 (0.88-1.48)	2.19 (1.48-3.22)**	1.54 (1.10-2.14)*	2.02 (1.16-3.51)*
Elderly with CKD	2.68 (1.95-3.68)**	4.43 (3.01-6.52)**	1.57 (1.26-1.97)**	1.80 (1.40-2.31)**	4.20 (2.78-6.35)**	2.55 (1.82-3.56)**	2.09 (1.26-3.49)*
Elderly with CKD *vs* Healthy elderly	1.22 (0.89-1.67)	2.69 (1.93-3.75)**	1.59 (1.26-2.01)**	1.57 (1.21-2.04)*	1.92 (1.34-2.76)*	1.66 (1.17-2.34)*	1.04 (0.57-1.88)
Albumin	-	-	0.64 (0.41-1.00)*	0.84 (0.49-1.45)	-		
Total bilirubin	-	-	-	0.76 (0.59-0.97)*	-		
C_{max} (pg/mL)							
Healthy young adults	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Healthy elderly	1.83 (1.40-2.38)**	1.23 (0.90-1.68)	1.12 (0.85-1.47)	0.97 (0.78-1.20)	2.28 (1.56-3.33)**	-	1.56 (0.97-2.50)
Elderly with CKD	1.86 (1.42-2.44)**	1.61 (1.17-2.21)*	1.90 (1.42-2.54)**	1.36 (1.10-1.68)*	4.42 (2.93-6.65)**	1.71 (1.26-2.33)*	1.57 (1.01-2.44)*
Elderly with CKD *vs* Healthy elderly	1.02 (0.77-1.34)	1.31 (0.95-1.80)	1.70 (1.31-2.20)**	1.40 (1.13-1.75)*	1.94 (1.35-2.78)**	-	1.01 (0.61-1.68)
Body mass index	-	-	0.93 (0.89-0.97)*	-			
Total bilirubin	-	-	-	0.62 (0.40-0.94)*			
$T_{1/2}$ (hr)							
Healthy young adults	1.00	1.00					
Healthy elderly	2.33 (1.40-2.38)**	1.22 (0.97-1.53)					
Elderly with CKD	2.56 (1.42-2.44)**	3.18 (2.49-4.06)**					
Elderly with CKD *vs* Healthy elderly	1.10 (0.80-1.50)	2.61 (2.07-3.30)**					

Data are presented in geometric mean ratio (95% confidence interval).

Variables with p value < 0.1 in the univariate analysis were included in the multivariable analysis.

[a]A subject with *SLCO1B1 wild-type*'s plasma drug concentration was an outlier and was excluded from the model but a subject with *SLCO1B1*15*17* was still included.

[b]Twenty four subjects with *ABCG2* variants (rs2231142, 421AA and 421CA) were excluded from the model.

*p <0.05, **p <0.001.

$AUC_{0\text{-last}}$: area under the concentration-time curve of time zero to the last time point; C_{max}: maximum plasma concentration; CKD: chronic kidney disease; $T_{1/2}$: half-life.

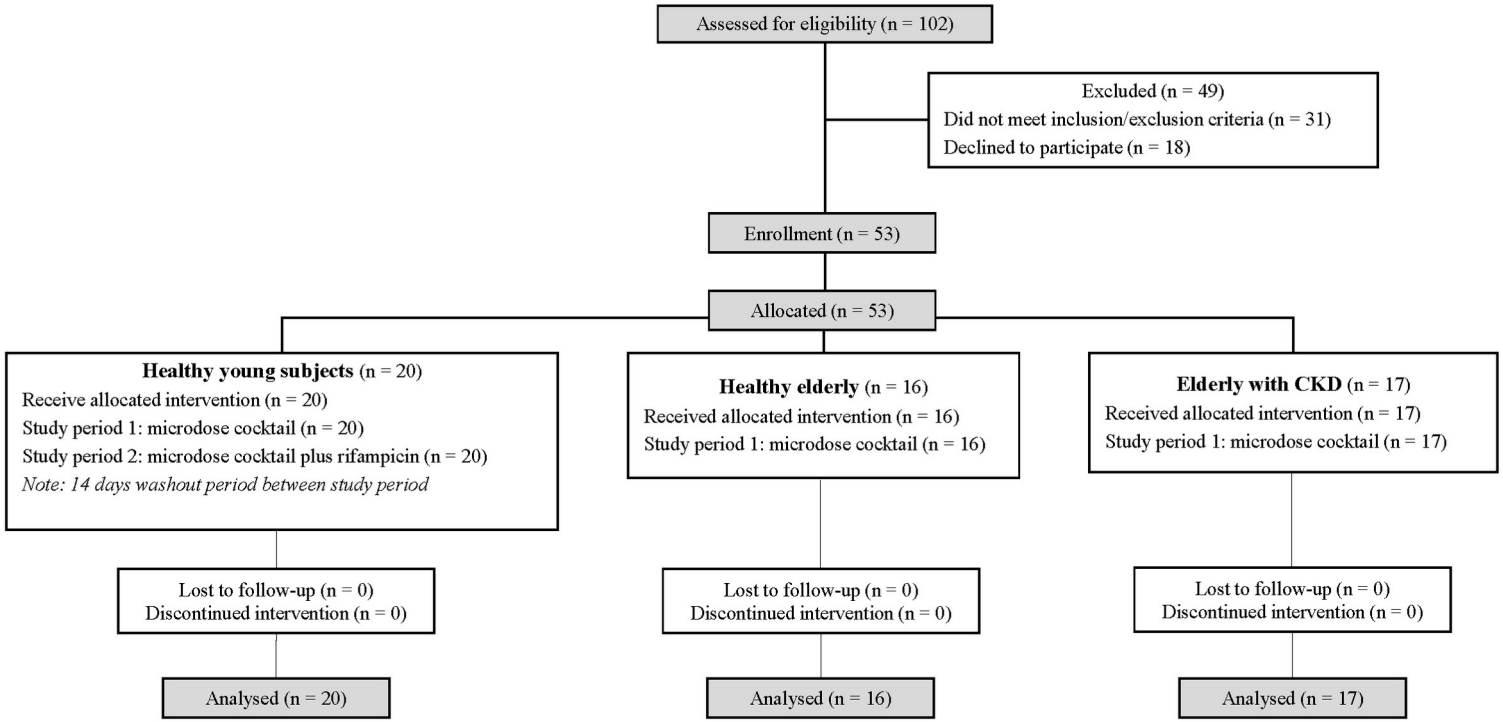

Figure 1 Study flow diagram.

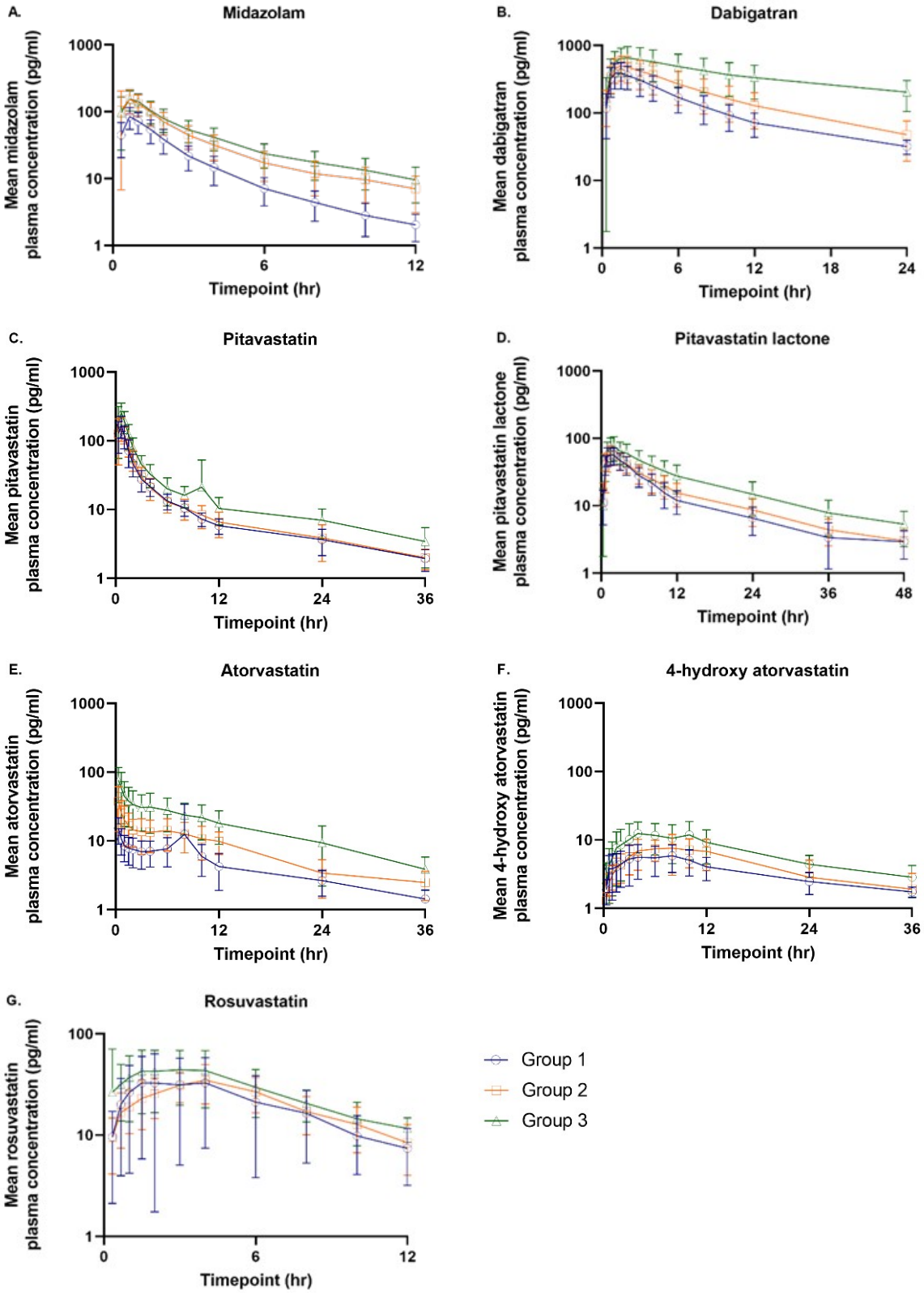

Figure 2 The plasma concentration-time curves of microdose cocktail probe substrates in 3 groups of participants.
Group 1: healthy young volunteers
Group 2: healthy elderly volunteers
Group 3: elderly patients with chronic kidney disease