PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Severity of Celiac Disease and Clinical Management Study When Using a CYP3A4 Metabolized Medication: A Phase 1 Pharmacokinetic Study
AUTHORS	Chretien, Marc; Bailey, David; Asher, Linda; Parfitt, Jeremy; Driman, David; Gregor, Jamie; Dresser, George

VERSION 1 – REVIEW

REVIEWER	Dr Prasad Neerati M.Pharm., Ph.D., PDF (USA). Assistant Professor of Pharmacy DMPK & Clinical Pharmacology Division Department of Pharmacology, University College of Pharmaceutical Sciences, Kakatiya University, Warangal, TS, India. Telefax: 08702453508 Cell:91-9494812120 E- Mail: prasadneerati@gmail.com
REVIEW RETURNED	16-Nov-2019

GENERAL COMMENTS	The research on "Severity of Celiac Disease and Clinical Management Study When Using a CYP3A4 Metabolized Medication: A Phase 1 Pharmacokinetic Study" tried to expalin the problem of celiac disease to alter the CYP3A4 activity. And the study design is good. Eventhough the authors are supposed to give the following questions to be answered properly. 1. How the pharmacokinetic parameters are estimated i.e the estimation protocols are missing they have to be answerd clearly. 2. What software is used to estimate the concentrations was it approved software. 3. Why not the metabolites are not estimated in the study. 4. Why the research articles are included at the end of the research article.

REVIEWER	Ida Robertsen Department of Pharmacy, University of Oslo
REVIEW RETURNED	10-Dec-2019

GENERAL COMMENTS	bmjopen-2019-0340086: Severity of Celiac Disease and Clinical Management Study When Using a CYP3A4 Metabolized Medication: A phase 1 Pharmacokinetic Study In the current study, Chretien et al investigate the felodipin pharmacokinetics in 47 patients with different severity in celiac disease. The authors also compare the findings to their own historical data (control group) in healthy subjects (total 68 subjects)

	where the interaction potential between felodipin and grapefruit juice has been assessed. The effect of celiac disease on the pharmacokinetics of felodipin is interesting and important. However, the inclusion of previous published data (healthy volunteers) as part of the results is in my opinion not the optimal way to present the results. In my opinion, the focus should be on the celiac patients. 1. In Figure 1, both the results from the current study in celiac patients and the historical data were included. Are the time-concentration profiles of felodipin obtained from the healthy volunteers paired data? If so, they should not be presented in the same figure as the concentration-time profile of felodipin from celiac patients. The felodipin concentrations from healthy volunteers were obtained between 15-20 years ago. There has been major improvements in the bioanalytical assays the last decade. 2. Although the historical data has been combined in the present manuscript, all of these data has previously published. This should be made clearer for the reader. The authors may consider leaving the historical data out of the results part and focusing on the celiac patients (which is the real novelty in this study). The historical data may be used to compare the results from the celiac patients in the discussion part of the manuscript. 3. The celiac patients are divided into four subgroups depending of the severity of their disease. All of the data analysis are performed on three groups. The authors may consider re-naming the groups to A, B and C instead of A, B+C and D for better readability. 4. Where the person performing the pharmacokinetic calculations blinded in relation to the different group of severity of celiac disease? 5. Why did the authors only sample the patients for 8 hr? The half-life of felodipin is approximately 25 hr. This should be included as a limitation of the study. 6. Please use the term exposure instead of concentrations. 7. The title does not reflect the content of the paper and are not specific. What do the authors refer to as “alternative clinical drug management”. Please revise. 8. P5, line 18-19: please change this sentence. It is not the CYP3A4 enzyme that changes the systemic drug concentration, but drugs inhibiting or inducing this enzyme and thus leading to a changed plasma concentration of substrate drugs. 9. In the conclusion of the abstract the authors may consider to be add that the increased felodipin concentrations were probably secondary to decreased small intestinal CYP3A4 expression as the authors have not shown this in their work, but it is the hypothesis for the observed change in felodipin exposure 10. Why did the authors include Table 3? I suggest that the authors remove this table from the manuscript, and refer the reader to reference 19 for this information. 11. The bioanalytical method of felodipin should be described in more detail. 12. Why did the authors choose 48 hr as their limit for intake of drugs that may potentially interact with CYP3A4? For drugs with a long half-life this may not have been sufficient. 13. The authors should consider adding more clinical data in Table 1. Did the group differ in total body weight or other inflammatory diseases that has shown to influence the activity of CYP3A?

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Reviewer Name: Dr Prasad Neerati
University College of Pharmaceutical Sciences,
Kakatiya University, Warangal, TS, India.

Please state any competing interests or state 'None declared': Nil

Response:
We have added (see AUTHOR CONTRIBUTIONSHIP) the statement:
'competing interests: none declared' for each author to clarify this concern.

1. How were the pharmacokinetic parameters estimated? The protocols are missing and they have to be answered clearly.

Response:

This was addressed in Methods (Pharmacokinetic analyses) as follows:
'Plasma felodipine concentrations were analyzed by the non-compartmental method. The terminal elimination rate constant (ke) was determined by log-linear regression (correlation coefficient of r >0.95 for the last 3 drug concentrations). The apparent elimination half-life (t ½) was calculated as 0.693/ke. Area under plasma drug concentration–time profile from 0 to 8 hours (AUC0-8) used the linear trapezoidal method. The AUC from 0 to infinity (AUC0-∞) was AUC0-8 plus AUC8-∞ with the later calculated by dividing the final plasma drug concentration by ke. Peak plasma drug concentration (Cmax) and the time to reach Cmax (tmax) were obtained directly from the experimental data.'

2. What software was used to estimate the concentrations? Was it approved software?

Response:

The first sentence of the above (Reviewer 1, Response 1) has been modified to read:
'Plasma felodipine and dehydrofelodipine concentrations were measured using the Microsoft program Excel 2016 and analyzed by the non-compartmental method.'

3. Why were the metabolites not estimated in the study?

Response:

We have metabolite data and are pleased to include them as requested in the manuscript.

Table 2 was extended to include dehydrofelodipine and dehydrofelodipine / felodipine ratio.

We have had to modify the Results to read:
'Plasma concentration – time profiles for felodipine are shown in Figure 1. Celiac patients in Groups A, B+C and D had statistically significant ($p<0.05$) linear trends for increasing felodipine and dehydrofelodipine AUCs and Cmax (Table 2).
Groups A and D had felodipine, dehydrofelodipine and ratio pharmacokinetic parameters that were comparable to those for healthy subject controls with water and grapefruit juice, respectively.
Healthy subjects had corresponding felodipine AUC 0-8, AUC0-∞ and Cmax with grapefruit juice (positive control) that were 280+25% ($p=0.0001$), 347+58% ($p=0.0001$) and 335+34% ($p=0.0001$) of those with water (negative control).'

Information was also added to the Discussion (2nd paragraph) to interpret the aspect of including pharmacokinetic metabolite data that states:
'Felodipine has the single primary metabolite dehydrofelodipine 9. The metabolism of both felodipine and dehydrofelodipine is mediated by CYP3A4 9. Increased felodipine and dehydrofelodipine AUC and Cmax, decreased dehydrofelodipine / felodipine AUC ratio and no alteration in tmax nor t ½ are consistent with lower presystemic CYP3A4 activity 9. The similar changes in felodipine, dehydrofelodipine and ratio pharmacokinetics for patients with severe celiac disease (Group D) and for healthy subjects with grapefruit (positive control) provide additional support consistent with the mutual mechanism of diminished small intestinal CYP3A4 expression that is known to occur in these two circumstances 6,7.'
4. Why were the research articles included at the end of the research article?

Response:

These are two publications of our previous work that we felt might provide useful background information to assist in the review of the manuscript.

Reviewer: 2
Reviewer Name: Ida Robertsen
Department of Pharmacy,
University of Oslo

Please state any competing interests or state 'None declared': None declared

Response:

Please see answer to Reviewer 1 on this matter.

1a. The inclusion of previous published data (healthy volunteers) as part of the results is in my opinion not the optimal way to present the results. In my opinion, the focus should be on the celiac patients.

Response:

We have re-arranged the Results section (see Reviewer 1, Response 3) so that the information about healthy volunteers comes at the end in an attempt to improve the requested better focus on the celiac patients.

Notably, the healthy volunteers played an important function by being negative and positive controls for the celiac patients by affording a large data base to be compared against the celiac patient population. A key deduction from this evaluation was the mutual mechanism of the pharmacokinetic interaction. Accordingly, drugs known or predicted to be affected by grapefruit would be similarly affected by patients with celiac disease.

The healthy volunteers also enabled the estimation of relative risk of an adverse drug event among the groups of celiac patients. Healthy individuals (given water) and celiac patients with the mildest form of this disease (Group A) would likely have comparable risk. On the other hand, healthy individuals (given grapefruit juice) and celiac patients with the severe form of the disease (Group D) would be at equivalently greater risk.

These findings were original, important and dependant upon the data obtained from the healthy volunteers.

1b. In Figure 1, both the results from the current study in celiac patients and the historical data were included. Are the time-concentration profiles of felodipine obtained from the healthy volunteers paired data? If so, they should not be presented in the same figure as the concentration-time profile of felodipine from celiac patients.
Response:

The time – plasma felodipine concentration profiles of the healthy volunteers were from crossover studies that involved paired data statistical analysis. However, Figure 1 only presents the mean + standard error profiles for the five treatment groups. We cannot think of any scientific or statistical reason why this would be inappropriate. Conversely, it provides an important perspective on the pharmacokinetic profiles among the five groups.

1c. The felodipine concentrations from healthy volunteers were obtained between 15-20 years ago. There have been major improvements in the bioanalytical assays the last decade.

Response:

This is a valid comment but it is not applicable in this situation. We have had great experience with the reported methodology. The analysis is automated, involves easy and complete extraction of felodipine and dehydrofelodipine from plasma and has the required sensitivity and selectivity for measuring the clinical plasma drug concentrations accurately. We have used it for all the individuals (healthy volunteers and celiac patients) involved in this investigation and saw no reason for change.

2a. Although the historical data has been combined in the present manuscript, all of these data have been previously published. This should be made clearer for the reader.

Response:

The Methods presents this information (Healthy Subjects, Study Population and Experimental Protocol). The Discussion (last paragraph) addresses the possible limitation of including previously tested healthy subjects. Thus, we feel that we have made it appropriately clear that these data have been previously published and that we have addressed possible concerns.

2b. The authors may consider leaving the historical data out of the results part and focusing on the celiac patients (which is the real novelty in this study). The historical data may be used to compare the results from the celiac patients in the discussion part of the manuscript.

Response:

We feel that the historical data played an important role (please see Reviewer 2, Response 1a).

3. The celiac patients are divided into four subgroups depending on the severity of their disease. All of the data analyses are performed on three groups. The authors may consider re-naming the groups to A, B and C instead of A, B+C and D for better readability.

Response:

Thank you for this suggestion. However, we feel that it is clearer to the reader when we present this group as B+C rather than just B to avoid possible confusion about its composition.

4. Were the person performing the pharmacokinetic calculations blinded in relation to the different

group of severity of celiac disease?

Response:

This is an important point. The following statement has been added (Methods, Pharmacokinetic analysis):
'The author (DGB) who calculated the pharmacokinetic results was blinded to the histological reports.'

5. Why did the authors only sample the patients for 8 hr? The half-life of felodipine is approximately 25 hr. This should be included as a limitation of the study.

Response:

This pharmacokinetic interaction investigation was devoted to examining differences in the oral bioavailability of felodipine. This would occur in the absorption phase of the plasma felodipine concentration – time profile.

Table 2 shows that the mean peak plasma felodipine concentration (Cmax), i.e. the maximum rate of absorption of drug from the gastrointestinal tract, occurred between 3.0 – 3.4 hours after dosing among all the groups. The plasma felodipine concentration decreased with a mean half-life (t ½) of 2.6 – 4.7 hours. Felodipine Cmax and AUC0-8 were proportionally higher to the same extent than their corresponding baseline value. Thus, the 8-hour time frame for this study was sufficient to quantify differences in oral felodipine bioavailability among the treatment groups.

Additionally, the 8-hour time frame was used as the standard in many of our other felodipine interaction bioavailability peer reviewed publications.

Conversely, obtaining plasma samples for drug analysis beyond the 8-hour period may raise the ethical issue of unnecessarily prolonging the duration of human testing.

We are also not clear from where the reviewer got the information of felodipine having a t ½ of 25 hours as no reference was provided.

6. Please use the term exposure instead of concentrations.

Response:

We measured drug concentrations and that is an accurate representation of what was reported. We are not clear why the reviewer has requested this change to the term 'exposure', which is not as exact.

7. The title does not reflect the content of the paper and are not specific. What do the authors refer to as "alternative clinical drug management". Please revise.

Response:

The title outlines that it was conducted in celiac patients, that the impact of severity of the disease was assessed and that it was a phase 1 pharmacokinetics investigation. We feel that this accurately represents what was done.

The phrase 'clinical drug management' is standard terminology for most English – speaking

healthcare professionals. Similarly, ‘alternative’ means ‘other’. We have made this substitution in the abstract in order to reduce the possibility of confusion in the broader reader population.

8. P5, line 18-19: please change this sentence. It is not the CYP3A4 enzyme that changes the systemic drug concentration, but drugs inhibiting or inducing this enzyme and thus leading to a changed plasma concentration of substrate drugs.

Response:

This sentence has been altered to read: ‘Thus, it plays an important role in many drug interactions that result from inhibition or induction of CYP3A4 that might thereby change systemic drug concentration and associated clinical response.’

9. In the conclusion of the abstract the authors may consider adding that the increased felodipine concentrations were probably secondary to decreased small intestinal CYP3A4 expression as the authors have not shown this in their work, but it is the hypothesis for the observed change in felodipine exposure.

Response:

Added as the first sentence of the abstract in Conclusions is the statement: ‘Increased felodipine concentrations in celiac patients were most probably secondary to decreased small intestinal CYP3A4 expression.’

10. Why did the authors include Table 3? I suggest that the authors remove this table from the manuscript, and refer the reader to reference 19 for this information.

Response:

Table 3 is an abbreviated version of what is found in reference 19. It presents just the more commonly prescribed drugs with the potential for a serious adverse event, in some cases life – threatening.

Obtaining the full article requires a subscription to the yearly updated publication ‘Compendium of Pharmaceuticals and Specialties’ by the Canadian Pharmacists Association. Consequently, readers may have difficulty obtaining it if they wanted the complete listing.

We were aware that the BMJ and BMJ Open were particularly interested in studies that could readily translate research findings into everyday clinical practice. Thus, we tried to tailor this manuscript to this purpose. Table 3 is a key aspect of this request.

The updated version (Sept 2019) of reference 19 is included as a supplementary file.

11. The bioanalytical method of felodipine should be described in more detail.

Response:

This methodology was detailed in several of our other publications. Nevertheless, it has now been included in this revision as requested (Methods, Assay of plasma felodipine and dehydrofelodipine concentrations):
‘Plasma (500 µL) was extracted with toluene (500 µL) containing the internal standard (H165/04; AB Haessle, Gothenburg, Sweden) by gentle oscillation of the mixture overnight followed by centrifugation. The toluene phase (1 µL) had split-less injection into a dual-tapered deactivated glass

insert (Hewlett Packard Canada Ltd., Toronto, Ontario, Canada) in a Hewlett Packard 5890 Series II Gas Chromatograph (Hewlett Packard Canada Ltd) equipped with a 63Ni electron capture detector and a 25-m x 0.32-mm inner diameter fused silica capillary column coated with a stationary phase of methyl silicone, 0.52-um (HP-1; Hewlett Packard Canada Ltd.). After a purge time of 1 min, the initial oven temperature of 90°C was increased at 30°C/min to 180°C. This was then increased at 5°C/min to 260°C that was held for 3 min. Next, the temperature was increased at 30°C/min to a final temperature of 280°C that was held for 5 min. The injector port and detector temperatures were maintained at 260°C and 300°C, respectively. The carrier gas was ultrapure helium (column inlet pressure of 100 kPa), and the make-up gas was ultrapure nitrogen (60 mL/min). The retention times of felodipine, dehydrofelodipine and internal standard were 20.1, 14.5, and 21.7 minutes, respectively. The coefficients of variation for plasma felodipine and dehydrofelodipine concentrations were 4.7% and 2.9% at 1.0 ng/mL (n = 5). The limit of detection was 0.25 ng/mL for both.'

12. Why did the authors choose 48 hr as their limit for intake of drugs that may potentially interact with CYP3A4? For drugs with a long half-life this may not have been sufficient.

Response:

There appears to be a need for clarification. It is stated in the Methods (Celiac Disease Patients, Experimental Protocol):
'They avoided consumption of any substance(s) which could have an impact on bioavailability of felodipine, including grapefruit, Seville orange (marmalades), lime, pomelo, tobacco, alcoholic drinks, medications (prescription and over-the-counter) and natural health products for at least 48 hours before and during course of this study.'

This 48-hour interval was included in the study design so that there would be sufficient time for the return of intestinal CYP3A4 activity to baseline before clinical testing in the event that an inhibitor or inducer might have been consumed. This mirrored the technique used in our previous grapefruit – drug interaction studies. Grapefruit, as well as related foods, cause irreversible inactivation of intestinal CYP3A4. Thus, it takes time for de novo synthesis of this enzyme. We reported that the half-life of recovery of intestinal CYP3A4 activity after ingestion of grapefruit juice approximated 12 hours (Bailey DG, Arnold JMO, Spence JD. Grapefruit juice-drug interactions. Br J Clin Pharmacol 1998; 46: 101-110). Thus, the 48-hour interval would enable intestinal CYP3A4 activity to be within 5% of baseline.

Additionally, we have added the following (Methods, Celiac Disease Patients, Experimental Protocol):
'All medications taken by the celiac patients were documented before entry into the study. None were receiving a medication that was either an inhibitor or inducer of CYP3A4 and had an elimination half-life of sufficient duration to have a relevant clinical effect on intestinal CYP3A4 after a 48-hour interval.'

13. The authors should consider adding more clinical data in Table 1. Did the group differ in total body weight or other inflammatory diseases that has shown to influence the activity of CYP3A?

Response:

Thank you for the suggestion. Table 1 has some basic background information about celiac patient population among the three groups. We thought that it would be helpful to the reader to find it in tabular form rather than to search for it in the text of the Methods. The purpose was not to provide a detailed demographic profile as the sample size within each group would be inadequate to make any statement about causality concerning total body weight or concomitant inflammatory disease.

VERSION 2 – REVIEW

REVIEWER	Dr PRASAD NEERATI KAKATIYA UNIVERSIT INDIA
REVIEW RETURNED	20-Jan-2020

GENERAL COMMENTS	Adequate changes have been made and acceptable, now this research article may be accepted for publication.

REVIEWER	Ida Robertsen Department of Pharmacy, University of Oslo
REVIEW RETURNED	30-Jan-2020

GENERAL COMMENTS	Thank you for the revision. I have no further comments.