Author's response to reviews

Title: Comparative Absorption of Curcumin Formulations

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Author's response to reviews: see over
The authors would like to thank the reviewers for their insightful comments on the paper, as these comments led us to an improvement of the work. Our revisions reflect all reviewers’ suggestions and comments. Detailed responses to reviewers are given below.

Reviewer’s (Jose Antonio) report:
No comments. The paper is rather blasé. I imagine there are folks who are interested in this very esoteric topic.

Reviewer’s (Dong Liang) report:
The authors presented a cross-over, 4-period pharmacokinetic study evaluating the apparent absorptions of curcuminoids from 4 curcumin formulations. In general, the experiment was well executed, and the data was clearly interpreted. The followings are specific comments for the authors to address with respect to their studies:

1. The pharmacokinetic sampling of 0-12 hours was a deficiency in the study design. Plasma levels of the conjugated curcuminoids were not in their elimination phases. Curcurmin half-life was estimated to be 6-7 hours in humans, thus at least 24 hour sampling is needed for pharmacokinetic characterization. Authors may want to discuss this point briefly in the Discussion section.

   We have added the following to the Discussion section: “One limitation in the study design was the sampling time frame. Our data indicated that the Curcumin half-life was estimated to be 6-7 hours and that the plasma levels of the conjugated curcuminoids were not in their elimination phase. Thus, while we sampled from 0-12 hours, we propose future research to assess a 24 hour sampling period.”

2. The LC-MS/MS method was referenced from a prior study by Cuomo [Ref#26], who adopted an assay method by Liu [Ref# 21]. The method by Liu is for rat plasma, not for human plasma. Thus, at minimum, the authors should provide their validation data which includes linearity, precision and accuracy. Furthermore, it is a concern to this reviewer that the standard curves consisted only 3 concentration points.

   We have added our validation data to the revised manuscript: “A six-point calibration curve was created by plotting the peak area ratio (y) of curcumin to internal standard versus the curcumin concentration. The regression parameters were calculated using the MassHunter Workstation Software (Agilent Technologies, Santa Clara, CA). The calibration curves were linear in human plasma with curves of y=1.24x (r=.99) and y=0.58x (r=0.99) for curcumin and tetrahydrocurcumin, respectively. The accuracy of curcumin and tetrahydrocurcumin in the control sample was 92-100% and 101-105%, respectively, with a coefficient of variation of 5.7 and 3.7%, respectively.”

3. Table 1 (was miss labeled in the text) should only show the MRM ion pairs used for the quantification, i.e., curcumin m/z 369.1 --285.1; tetrahydrocurcumin m/z 373.2 --137.1, and so on.

   Table 1 has been removed and “the transitions monitored were m/z 373.2 → 137.1 for tetrahydrocurcumin, 369.1 → 285.1 for curcumin, 339.1 → 255.1 for demethoxycurcumin, and 309.1 → 225.0 for bisdemethoxycurcumin.” has been added to the text.
4. "Statistical Analysis" indicated a Nonlinear Mixed Effects Model of population pharmacokinetic analysis, but the model analysis was not presented in the Result section. We have added the model analysis to the Results section: “The fixed effects were the curcuminoids, formulations (products), the interaction curcuminoids * formulations, and the interaction of curcuminoids * formulations * time. The data model is a population model with both random slopes and intercepts where the slopes (third-order polynomial functions) and intercepts vary among the subjects to estimate the parameters in the entire population. The time points 1-12 were including in the analysis but not including the time point 0 that was all 0 ng/mL so the random intercepts were time point 1. The addition of parameters were compared by the Information Criteria log likelihood function and the significance values for adding/removing parameters based on the specific number of degrees of freedom (df). The final model was Restricted Maximum Likelihood (RML).”

5. "Sample Preparation": "...and 50 uL of methanol" should be removed.
   “and 50 uL of methanol” has been removed.

6. In the Result section, "Figure 2" should be "Figure 4 & 5", and "Table 1" should be "Table 2".
The figures and table in the Results section have been adjusted accordingly.

7. The authors should provide more discussion with respect to significantly increased oral absorption (45.9-fold) of total curcuminoids from the CHC formulation as compared with the CS formulation. What was the reason of this improvement? Was an increased solubility of curcuminoids in the GI tract, or improved permeability in the GI tract due to the excipients in the CHC formulation, or potential inhibition of the transporters?
The increased oral absorption of the CHC formulation as compared with the CS formulation is based on an increased solubility of the CHC formulation. The solubility was enhanced by dispersing a highly purified powder [with min 95% curcuminoids] in a water-soluble carrier (polyvinyl pyrrolidone) along with other encapsulating agents. Tocopherol and ascorbyl palmitate were used to prevent degradation of curcumin. This information has been added to the Discussion section of the revised manuscript.

8. "Figure 5" legend: Please indicate dose levels.
Dose levels have been added to the legend of figure 5.