Reviewer’s report

Title: Safety, Tolerability, and Pharmacokinetics of Repeated Oral Doses of 2-Hydroxybenzylamine Acetate in Healthy Volunteers: A Double-blind, Randomized, Placebo-controlled Clinical Trial.

Version: 0 Date: 31 Oct 2019

Reviewer: Scott Weir

Reviewer's report:

Overall, this early phase clinical trial was well designed and conducted by the excellent clinical pharmacology program at Vanderbilt. My questions, comments and suggestions are as follows:

1) Did the investigators prepare and submit an investigational new drug application to FDA? If so, mention in the manuscript. If not, explain why not.

2) Did the manufacturer provide 2-HOBA drug product dissolution and content uniformity data? If not, did the investigators perform CU on drug product? If not, why not? What acceptance criteria were utilized for the product tested?

3) The rationale for Q 8 hr dosing is not clear. The authors describe the apparent elimination half-life, based on single dose data, is ~ 2 hours. The manuscript also describes that 2-HOBA is not detectable in plasma after 8 hours, yet, Q 8 hr dosing was selected to "maintain effective plasma levels". This needs to be clarified in the manuscript prior to publishing.

4) Given 2-HOBA was administered orally, and absolute bioavailability is not known, nor are there data ruling out dose-dependent bioavailability, greater clarity needs to be incorporated into the manuscript that the volume of distribution and clearance estimates are not true measures of Vd and Cl.

5) The description of urine sample collection is not clear. Did the investigators collect clean catch samples at 4, 8, 12 and 24 hours or complete urine samples via urine collection intervals (e.g., 0-4, 4-8, 8-12 and 12-24 hr)? It's not clear from the data presentation.
6) Please describe in this manuscript under what conditions the plasma bioanalytical methods for 20-HOBA and salicylic acid (SA) were validated. As well, please describe the in-process performances of the two assays during routine analysis of samples.

7) The manuscript lacks a statement on whether the serial blood (plasma) PK sampling scheme utilized is sufficient given the PK of 2-HOBA and SA. Only 7 samples were collected over the dosing interval. It would be helpful to see plasma drug and metabolite concentration-time profiles presented as semilog plots to see whether, post Tmax, the declines in plasma drug/metabolite concentrations were mono- or bi-phasic. From the data presented, it is difficult for the reader to interpret whether the half-lives represented apparent elimination phases.

8) Please clarify for the editor whether accumulation ratios were calculated correctly. If I just look at the mean data presented in the PK table for 2-HOBA, I get AR's of 1.189 and 1.32 for the 500 and 750 mg dose groups, respectively. These values are lower than the within subject data. The description "mild" 2-HOBA accumulation" in the results/discussion section and "did not cause excessive accumulation" are not useful descriptions of accumulation. I'd like to see the authors focus on whether single dose pharmacokinetics predict steady-state pharmacokinetics. The descriptions imply or suggest accumulation is a bad thing.

9) Given the half-life and Q 8 hr dosing, please include in the 2-HOBA and SA tables average steady-state concentrations (over the dosing interval) as well as percent fluctuation parameters.

10) The similar drug exposures for the 500 and 750 mg doses are attributed by the authors to be due to considerable inter-subject variability. Is it possible 2-HOBA exhibits dose-dependent absorption?

11) Lastly, and related to comment (10) immediately above, the authors attribute the drastic differences in CSF/plasma ratios for 2-HOBA and SA to protein binding. This is a reasonable conclusion, however, I would like to see a discussion or at least consideration for pKa differences for parent and metabolite. Is it possible that the degree of ionization in plasma contributes to the differences? If unlikely, explain why.
Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

Yes

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

Yes

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics

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