Reviewer's report

Title: (R)-[11C]Verapamil PET studies to assess changes in P-glycoprotein expression and functionality in rat blood-brain barrier after exposure to kainate-induced status epilepticus

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Reviewer: Mark Muzi

Reviewer's report:

Comments on the manuscript “(R)-[11C]Verapamil PET studies to assess changes in P-glycoprotein expression and functionality in rat blood-brain barrier after exposure to kainate-induced status epilepticus”. Reviewed by Mark Muzi and Sara Eyal.

Synopsis: The authors describe a PET and IHC study of P-gp expression and function at an early time point in the kainate model for spontaneous seizures in rats. NONMEM analysis detected small differences between kainate-treated and control rats in verapamil BBB kinetics when P-gp was inhibited. The manuscript is concisely written and the results are of interest, but there are concerns about the modeling approach and the major conclusion, as described below.

Major comments:

1. The paper evaluates too many models in light of the available biological data to select among them. Previous publications indicate that a simple one-tissue compartment model for the initial 10-15 minutes determining the transfer constant K1 assess P-gp activity with verapamil and avoids contamination of brain regions with radiometabolites (Muzi 2009, Ikoma 2006, both in J Nucl Med, and the review by Kannan 2009 in Clin Pharmacol Ther). Measures of verapamil retention in tissue (Logan distribution volume, for example) may have nothing to do with P-gp activity at the surface of the BBB, unless there is significant and persistent binding (infinite binding) of verapamil entering brain tissue throughout the 60 minutes of scanning to track the transfer into brain. Please indicate the biological basis for selecting each of the models presented in this paper and justify the use of 60 minutes of imaging data overall, but specifically for the 1C model.

2. The study evaluated P-gp expression and function at a single time point after kainate injection. The lack of change in P-gp expression / functionality on day 7 may reflect selection on a non-representative time point or inability of the study methodology to detect a change in P-gp rather than a stable level of expression (in the absence of a positive control). Therefore, the results appear to be preliminary and do not necessarily lead to the general conclusion that P-gp expression and activity do not seem to change at an early stage after kainate treatment.
3. Could regional changes in P-gp activity (such as in the hippocampus) be detected when whole brain VOIs are evaluated? The major concern is that significant changes in P-gp activity in relevant brain regions may be masked by the overall unaffected P-gp activity in the rest of the brain.

4. P. 8, second paragraph: The 10 blood samples add up to 1 mL for sample activity plus 0.9 mL from three blood samples for metabolite analysis, and assuming some waste in catheterization and clearing lines between samples this totals potentially 3-4 mL of lost blood from the animal, which is significant for a 200g rat. Assuming about 12 mL of blood in a 200g rat, how does a loss of 20-30% blood volume affect delivery and metabolism of verapamil.

5. P. 11, second paragraph: Vb (vascular volume of brain) should be floated in compartmental models and not subtracted prior to curve fitting, as this would likely affect K1 determination.

6. P. 13, lines 13-14: AIC comparisons cannot be made due to violation of the assumptions of the Akaike criteria (differences among the models in parameter numbers). Additionally, the AIC does not indicate the biological basis for model selection.

7. The Methods section does not describe the statistical tests used for between-group comparisons. Given the 4 treatment groups, the appropriate test is ANOVA and not t-test.

8. Background: although it has been established that P-gp is over-expressed at the BBB of patients with pharmacoresistant epilepsy and in animal models of epilepsy, there is no clear evidence that p-glycoprotein limits brain uptake of antiepileptic drug or contributes to AED resistance. Also, the association between increased functionality of BBB efflux transporters and disease development or severity (as described in the abstract) is not supported by experimental data from the references cited in the Background section.

9. Consider citing the recent article by Bartmann et al. (Epilepsia 2010 Jul 14).

Minor comments:

1. Title: Please delete [0] between “expression” and “and” in the title.

2. Abstract, Methods: verapamil is the most established PET ligand for determining BBB P-gp functionality, but not necessarily the best, in particular when studying over-expressed BBB P-gp.

3. Background, P. 4, line 6: patients are normally treated at maximal tolerated dose and not level.

4. Background, P. 4, line 5 from page end: the BBB P-gp expression is upregulated.

5. Methods, What was the PET image resolution?
6. Methods, P. 8, lines 13-15: For iterative reconstructions, like OSEM, the authors should indicate the number of subsets (s) and iterations per subset (i) performed or the number of updates (s * i = update number). Please provide the post-reconstruction filtering parameters.

7. Methods, Data analysis, P. 11: It would help the reader if the sentence beginning with “liver, obtained from the PET images” is re-written such that the content is more clear.

8. Methods, P. 10, line 15: I assume that Milli Q is ultrapurified water from Millipore’s Milli Q system, but that should be stated more directly.

9. Results, P. 13, line 4: Models are fitted to the data and not vice versa.

10. Discussion, P. 21, second paragraph: The words “of (R)-[11C]Verapamil” should follow “co-administration”.

11. Please check the equations and parameter definitions. For example, Vbr2 and Q2 (Fig. 3 and text) are not defined. Is the determination of Kp for Logan, and the other models identical? All parameters used in the paper should be defined. Consider a table of parameter definitions and formulas.

12. Fig. 5: Consider deleting the figure because it does not add to the data described in the text or on Fig. 6. In addition, the legend does not specify the treatment group or the brain region.

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:
'I declare that I have no competing interests'