Reviewer's report

Title: Antibodies against gonadotropin-releasing hormone (GnRH) and destruction of enteric neurons in 3 patients suffering from gastrointestinal dysfunction

Version: 2 Date: 26 December 2009

Reviewer: Tamas Ordog

Reviewer's report:

GENERAL COMMENTS

This revised manuscript investigates the role of circulating autoantibodies against gonadotropin-releasing hormone (GnRH) in neuron degeneration in the gut and consequent dysmotility in 3 patients with complicated gastrointestinal dysmotilities. The authors made substantial changes in response to the reviewers’ comments but were unable to provide evidence supporting a causal role for anti-GnRH autoantibodies in the observed neuron loss. Below is a detailed evaluation of the revised manuscript and the authors’ responses to the reviewers’ criticism.

EVALUATION OF THE AUTHOR’S RESPONSE

Reviewer 1 only offered congratulatory remarks. Responses to Reviewers 2 and 3 are discussed together.

1. Both Reviewers 2 and 3 noted that the data as presented did not provide conclusive evidence supporting a role for anti-GnRH autoantibodies in enteric neuronal degeneration. The authors were unable to produce such an evidence in the revised paper and considered this a limitation of the study. Unfortunately, this is not acceptable since the main conclusion of the paper is still that “Autoantibodies against GnRH may be an important trigger related to neuron degeneration and chronic gastrointestinal symptoms in some patients with intestinal dysmotility”. While it may be so, this study fails to prove it. As pointed out by the reviewers, data presented in Figure 3b do not, in any way, support a role for anti-GnRH antibodies as a factor limiting neuron survival. The results obtained with buserelin, a long-acting GnRH agonist, are even more confusing since in the pituitary, GnRH and its agonists act as functional antagonist when administered continuously (see classic works by Ernst Knobil and Jimmy D. Neil). Similar desensitization may also occur in other GnRH target cells. Thus, increased neuronal survival in this system may very well have occurred in the presence of downregulated GnRH receptor-coupled signaling pathways.

2. Missing figures have been replaced. However, the authors should have provided low-power GnRH and GnRH receptor immunohistochemistry images from a control and Case 3 to illustrate the dramatic decrease in positive neurons reported in the Results.
3. Case presentations: The authors expanded the description of the 3 cases. The text clearly conveys that these patients represented significant diagnostic and therapeutic challenges justifying their evaluation by unconventional diagnostic techniques.

4. CD40: The rationale for looking at circulating CD40 levels is now provided.

5. Use of primary cultures of dissociated longitudinal muscle – myenteric plexus preparations as an in vitro indicator system: This system certainly has significant limitations due to its inherent variability and the lack of a suitable denominator that could be used for the normalization of neuron counts. However, similar indicator systems have been used by other investigators and the results obtained in this study also appear reliable (albeit inconclusive). It should also be mentioned that the authors’ contention to that these primary cultures lack immune cells (see response to Reviewer 2) is most likely incorrect but the presence of such cells probably does not account for the observed results.

6. The 100% homology between rat and human GnRH is explained.

7. Controls for immunohistochemistry: Detailed description of the methods including appropriate controls is now provided.

EVALUATION OF THE REVISED MANUSCRIPT

1. Is the question posed by the authors well defined?
   Yes

2. Are the methods appropriate and well described?
   A key method (pre-absorption of anti-GnRH antibodies in patient serum) is lacking.

3. Are the data sound?
   The presented data are reliable but they are inconclusive at best.

4. Does the manuscript adhere to the relevant standards for reporting and data deposition?
   Yes

5. Are the discussion and conclusions well balanced and adequately supported by the data?
   The main conclusion of the paper is NOT supported by the results presented.

6. Are limitations of the work clearly stated?
   Yes. Unfortunately, these limitations are too great and make the results inconclusive.

7. Do the authors clearly acknowledge any work upon which they are building, both published and unpublished?
Yes

8. Do the title and abstract accurately convey what has been found?
No. The conclusion is not supported by the results.

9. Is the writing acceptable?
Needs minor editing.

Level of interest: An article of insufficient interest to warrant publication in a scientific/medical journal

Quality of written English: Needs some language corrections before being published

Statistical review: No, the manuscript does not need to be seen by a statistician.

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