Reviewer's report

Title: Nasal administration of the neuroprotective candidate NeuroEPO to healthy volunteers: A randomized, parallel, open-label safety study

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Reviewer: William H. Frey

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Nasal administration of the neuroprotective candidate NeuroEPO to healthy volunteers: A randomized, parallel, open-label safety study. This clinical trial found that NeuroEPO was a safe for treatment over a period of 4 days, was well tolerated at the nasal mucosa level and did not stimulate erythropoiesis in healthy volunteers.

* This manuscript needs to be improved by correcting the grammar used and by using more precise language and more appropriate references. For example, in the second paragraph of the Background section, the authors state the following:

"Such molecule might be administered by a non-systemic route, such as the intranasal. Because the nasal mucous is one of the most permeable areas, intranasal delivery provides a practical, noninvasive method of bypassing the blood-brain barrier (BBB) in order to deliver therapeutic agents to the brain. This method allows drugs that do not cross the BBB to be very fast delivered to the Central Nervous System (CNS) into few minutes [7, 8]."

In general, intranasal delivery is not considered a "non-systemic route" of administration since it often leads to delivery of a therapeutic agent into the bloodstream and from there to most of the organs of the body such as the heart, lungs, liver, kidney, muscles, etc. It is only delivery to the upper third of the nasal cavity to contact both the olfactory and trigeminal neural pathways that has been reported to effectively bypass the blood-brain barrier and target therapeutics to the CNS while reducing systemic exposure. Therefore, in order for the readers to understand what the authors intend, they need to first introduce this specific type of intranasal delivery and say a few words about its mechanism and advantages. The authors' statement that "Because the nasal mucous is one of the most permeable areas, intranasal delivery provides a practical, noninvasive method of bypassing the blood-brain barrier (BBB) in order to deliver therapeutic agents to the brain." is not correct. If the nasal mucosa is highly permeable, it might explain why a protein therapeutic agent would rapidly enter the blood and systemic circulation, but it does not explain why it bypasses the blood-brain barrier.
I recommend that the authors clearly state or reference in their review some of the following key discoveries made by investigators in this field, including the discovery of the intranasal method for bypassing the blood-brain barrier and how this method of delivery and CNS targeting works.

1. In 1989, William H. Frey II, Ph.D. first discovered the non-invasive intranasal method that bypasses the blood-brain barrier to deliver and target therapeutics (including therapeutic proteins) along the olfactory neural pathway to the brain to treat neurodegenerative disorders such as Alzheimer's and stroke and later expanded the specific use of intranasal insulin to treat Alzheimer's disease. (See: U.S. Patents 5,624,898; 6,313,093 which are available online and contain approved and issued claims covering intranasal delivery of neurologic therapeutic agents to the brain and specifically the use of intranasal insulin to treat Alzheimer's disease.) Also see:


Yue-Ping Yu, Qiu-Qin Xu, Qi Zhang, Wei-Ping Zhang, Li-Hui Zhang, Er-Qing Wei (2005) Neuroscience Letters 387 5-10 Intranasal recombinant human erythropoietin protects rats against focal cerebral ischemia.

2. Dr. Robert Thorne first demonstrated that intranasal therapeutics also reached the brain from the nasal mucosa by traveling along the trigeminal neural pathway. [See: RG Thorne, GJ Pronk, V Padmanabhan, WH Frey (2004) Neuroscience 127 (2), 481-496. Delivery of insulin-like growth factor-I to the rat brain and spinal cord along olfactory and trigeminal pathways following intranasal administration.]

3. The authors need to read and also reference the following paper about the mechanism of intranasal delivery of drugs to the brain. [JJ Lochhead, DJ Wolak, ME Pizzo, RG Thorne (2015) Journal of Cerebral Blood Flow & Metabolism 35 (3), 371-381. Rapid transport within cerebral perivascular spaces underlies widespread tracer distribution in the brain after intranasal administration.] By reading this paper, the authors and the readers of their review, which can reference this article, will have a much better understanding of the pathways and mechanism by which drugs reach the brain from the nose.

* The above suggested references have a direct impact on this manuscript and the interpretation of its findings. For example, the paper cited above by Alcalá-Barraza et al. demonstrates that intranasal EPO not only rapidly reaches many brain regions but does so in part by traveling along the trigeminal neural pathway. The involvement of the trigeminal pathway in delivery from the nasal mucosa to the brain is important for this manuscript since the trigeminal nerve is the primary nerve involved in headache, which the authors state in the Abstract is the most reported adverse event. The authors also state that "Only two events (headache and epicondylitis), both in patients from Group A, were classified as grade 2 (moderate)." Note that 88.6% of the adverse events reported in this manuscript did not require treatment, but it was necessary to administer dipyrone to treat headache. Nasopharyngeal itching was another symptom likely to have been caused by the product. As reported by O. Pfaar et al (2009) in Pathophysiology of itching and sneezing in allergic rhinitis. SWISS MED WKLY 139(3-4):35-40, "Sensory nerves of the afferent trigeminal system including myelinated Aδ-fibres and thin, non-myelinated C-fibres of the nasal mucosa transmit signals generating sensations, including itching and motor reflexes, such as sneezing." It is therefore not surprising that intranasal NeuroEPO which is shown by the references I provided above to be transported directly from the nasal mucosa to the brain along the trigeminal neural pathway might cause the side effects of headache and itching of the
nasopharynx. Similarly, any of the formulation components might also cause these adverse side effects since they would also likely be transported along the trigeminal neural pathway. (For more on this, see below.)

* The intranasal delivery references cited above are more relevant to this paper than those provided in the paper, refs (7,8), when it comes to the discovery, pathways and mechanisms involved in direct intranasal delivery of neuroEPO and other therapeutics to the brain. This includes the following reference: Yue-Ping Yu, Qiu-Qin Xu, Qi Zhang, Wei-Ping Zhang, Li-Hui Zhang, Er-Qing Wei (2005) Neuroscience Letters 387 5-10 Intranasal recombinant human erythropoietin protects rats against focal cerebral ischemia.

* The manuscript does not explain how the authors know that the adverse events of headache, itching, etc. are not simply caused by the formulation components rather than by neuroEPO. These formulation components include: buffer salts, polysorbate 80, sodium EDTA, NaCl, benzalkonium chloride, HPMC F4M. Recognize that the dose of the formulation components is not the same in Group A and Group B. Rather, the dose of the formulation components is twice as high in Group A. This is a significant problem for interpretation of the results. Without additional controls, it is difficult to know whether the formulation components or the NeuroEPO is responsible. The reviewer recognizes that these are not severe adverse events.

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.

Unable to assess

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

No

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
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