Author's response to reviews

Title: NG2 and phosphacan are present in the astroglial scar after human traumatic spinal cord injury

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Version: 2 Date: 3 March 2009

Author's response to reviews:

Re: BMC Neurology - MS: 1792994857236830 - NG2 and phosphacan are present in the astroglial scar after human traumatic spinal cord injury#

Dear Editor,

Thank you for the reviewers’ comments to the above manuscript. We found the constructive criticism most helpful and have done our best to address all the points raised and have included extra data. We believe that the revised manuscript is of a higher quality and hope that it is now acceptable for publication in BMC Neurology. Our responses to the reviewers’ comments are dealt with in detail below:

Reviewer Eve Tsai

Major Compulsory Revisions

1. The methods overall are appropriate and well described. A more detailed description of their grading system may help to clarify their results. This could be accomplished by providing a picture for each level of their grading system or a detailed description for each level of their grading system or a reference for their grading system. I note that in their table, that none contain “++++”. Therefore, it may not be necessary to have a category that is “+++++”. In the text, they indicate “+++++” is “large numbers of labeled cells” and in the table the indicate
that “+++++” is “maximum amount of cells”. If they could clarify what “maximum amount of cells” means, that might help improve the reader’s understanding and interpretation of the data.

Response:

We introduced a new figure showing representative stainings for each level of our rating scale (Fig.1). Furthermore, we left out the level ++++++, which is not necessary for the grading system.

2. NG2 has been shown to label both Schwann cells and oligodendrocyte precursor cells (OPCs). In their description, they indicate that there is regrowth or infiltration of Schwann cells. A description of how they determine that these cells are truly Schwann cells rather than OPCs or oligodendrocytes would be helpful.

Response:

The differentiation between NG2-immunopositive Schwann cells and oligodendrocyte precursor cells is certainly important. A previous study in the same human cases (Buss et al., Brain 2007) could not detect Schwann cells in the intermediate zone using an anti-NGFr antibody. Therefore, the stellate-shaped cells found in this area at survival times of 2-11 days after SCI most likely represent OPCs. We introduced a new paragraph about these earlier results (page 16-17). In the lesion epicentre we detected neither cell population to be NG2 immunopositive which might be due to technical difficulties as stated in the discussion section (page 17). We did detect NGFr positive Schwann cells in this area (Buss et al., Brain 2007). We cannot exclude the presence of OPCs in the epicentre. It appears however unlikely, as this area represents a PNS milieu being sealed off the surrounding CNS parenchyma.

Minor Compulsory Revisions

1. In the methods, there is description of the use of anti-NGFr, although I am not clear where they assessed this in their results.

Response:

We have excluded the passage about the anti-NGFr antibody. This was clearly an error on our part as we are not presenting NGFr data.

2. Some details which may improve the reader’s interpretation of the data would be to include when the spinal column was obtained after death in Table 1. If a
The specimen was obtained 48 hours post death, then the results of the immunohistochemical studies may be influenced differently compared to a specimen obtained within 15 hours. A specimen obtained 48 hours post death may have already undergone necrotic degeneration as the authors note in their discussion which may influence the immunohistochemical and architectural results. Knowledge of when the specimens were obtained post death would allow the reader to better interpret the data.

Response:
We introduced data on the post mortem fixation time in tables 1 and 2.

3. Definition of some of the abbreviations used in the text might improve the reader’s understanding (e.g. OPCs, NF, MBP, NGFr).

Response:
We included the explanation for the abbreviations in the text and in the Abbreviation section.

Discretionary Revisions
1. The authors have published other immunohistochemical studies on likely the same sample group in other journals including this one. It might be helpful to the reader to have the authors acknowledge their work in the text and reference the other articles to help guide the reader to their other studies performed on this sample of spinal cord.

Response:
We introduced our previous studies performed in the same group of human spinal cord injury specimen (page 20).

Reviewer: L Gerard Toussaint III

1. How far rostral and caudal to the lesion were samples collected?

Response:
Usually, the whole spinal cord was cut into blocks of about 1 cm thickness and embedded in paraffin. If not, the lesion site including the epicentre and the intermediate zone comprised 5-6 segments and was always prepared completely. Furthermore, blocks from C1/2, C6/7, Th2/3, Th8/9, L1/2 and L4/5 were investigated, taking into account the level of injury.

2. Why did the authors choose to study 4 proteoglycan molecules and not 5, 7, or 3? Brevican, perlecan, and others may be important. Some reference to the importance of these four over others seems reasonable to expect.

Response:

The number of antibodies which function reliably in human paraffin embedded tissue is limited. We tried antibodies against other members of the CSPG family including brevican and aggrecan, however the staining results were not sufficiently reproducible in our spinal cord cases. This may, for example, also point to your remark 5 about the half life of the different proteoglycans with the more stable proteins being studied in this investigation.

3. The authors present results in two groups of patients – early and late post-injury death. However, in the ‘late’ group, the narrative divides these into 24 days-4 months, and >4 months. Adding a third group (early, mid-range, and late) would not devalue the data and may make them easier to understand.

Response:

The time frame from 11 days up to 4 months is certainly one of the most interesting ones and we are unfortunately lacking tissue specimens apart from the 24 days case. As this mid-range group would consist only of 1 case we included it in the late survival times, even though the staining results clearly differ from the following 4 months case.

4. The authors may benefit from mentioning that macrophages staining positive for certain proteoglycan species may have positivity from phagocytosis as well as native production of these molecules. I did not see micrographs with enough resolution to determine which was more likely.

Response:

As we were not able to solve this important question we mentioned this limitation in the result section (page 12)
5. The determination of the half life of proteoglycans after death is central to the data. The authors address this shortcoming. An animal experiment could be proposed, at least, showing the temporal loss of NG2, neurocan, versican, and phosphacan with fixation 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, or 48 hours after death. Are these data already in existence?

Response:

We possess a large tissue bank of unlesioned spinal cord specimen with a wide variety of post mortem fixation times. Fixation times up to 48 hours after death did not result in apparent differences concerning the quality of immunohistochemical staining. We are not aware of a published animal experiment as is proposed in this chapter. In our lab, we did not perform such an investigation.

6. Conclusions about the role of each PG should be tempered. We have here multiple snapshots in time and space, but do not know about potential transient expression of various PGs at the exact time when axons are trying to regenerate. NG2 and phosphocan in the astroglial scar may be secreted to foster regeneration, but their function inhibited by an undetermined molecule. The location of their expression makes them potential candidates for further investigation on inhibitors of neuro-regeneration. Neurocan and versican in the lesion core are described as “unlikely to participate in failed regeneration.” We don’t know if these are secreted to foster regeneration and that effect is negated by some other mechanism. Their location makes them less likely candidates for further investigation. That’s the utility of this paper – to parse out what is more or less likely to be important based on location and timing of expression.

Response:

We always try not to over-interpret our data with this limited number of specimens of human spinal cord injury. Since your comments are correct, we have added a paragraph to include the limitations of our immunohistochemical approach (page 19).

We hope that the efforts we have made to comply with, or answer, the reviewers’
comments have been sufficient to improve the quality of the manuscript and that it is now acceptable for publication in BMC Neurology.

Sincerely yours,

Dr. Armin Buss