Author's response to reviews

Title: Reduction in Podocyte Density as a Pathologic Feature in Early Diabetic Nephropathy in Rodents: Prevention of by Lipoic Acid Treatment

Authors:

Brian Siu (bbsiu@umich.edu)
Jharna Saha (jhsaha@umich.edu)
William E Smoyer (wsmoyer@umich.edu)
Kelli A Sullivan (ksulliva@umich.edu)
Frank C Brosius III (frosius@umich.edu)

Version: 2 Date: 2 December 2005

Author's response to reviews: see over
To the Board:

It is our pleasure to submit our revised manuscript entitled, “Reduction in Podocyte Density as a Pathologic Feature in Early Diabetic Nephropathy in Rodents: Prevention by Lipoic Acid Treatment” for consideration for publication in BMC Nephrology. We wish to thank the editors and the reviewers for their careful consideration of our manuscript in BMC Nephrology. We have made virtually all the changes recommended by the reviewers and editors and have detailed them below.

We hope that this submission is now appropriate for BMC Nephrology.

Responses to Reviewers

Responses to Dr. Nelson

We greatly appreciate these comments and are grateful that our study was felt to “demonstrate a relationship between podocyte density and diabetes and nicely outline several threats to validity in [our] small study.” This reviewer’s major concern was about our suggestion that the relatively abrupt decline in podocyte number between 6 and 8 weeks in the diabetic rats was due to artifact. Consequently, Dr. Nelson felt that our conclusion that a gradual loss of podocytes occurs after the initial steep decline in the first two weeks of STZ diabetes contradicted our data. The authors are in agreement with Dr. Nelson on this point and perhaps overstated the potential lack of accuracy and precision of our methods. The only point we should make in this regard is to indicate that these data were obtained in separate trials, rather that in a single trial with multiple timepoints, and that therefore some unintended and unmeasured differences in experimental conditions could have contributed to these differences. We agree that these data do not support the notion of early podocyte loss followed by only a gradual decline. Indeed the decline in podocyte density and apparent number appears to continue at a significant rate at least through the first 8 weeks of STZ-diabetes.

More minor concerns are addressed in order below. 1) Why was lipoic acid used as an antioxidant? We chose this compound since it directly reduces mitochondrial superoxide production. Since enhanced mitochondrial ROS production has been implicated as the primary event leading to all microvascular diabetic complications, and therefore was an important target for a therapeutic intervention. This logic is now explicitly stated in the Discussion and we thank the reviewer for this suggestion.

2) Why include mouse studies? We included these data to demonstrate that similar methods could be utilized in mouse as well as rat models of diabetes since the mouse has become such an important species on which to study the impact of genetic manipulation.
Responses to Dr. DeRubertis

We also greatly appreciate these recommendations for revision and agree with virtually all of them. The first concern was that some of the podocyte damage could have resulted from streptozotocin (STZ) toxicity. This is an important concern and one that the authors have also shared. While our studies do not absolutely rule out the possibility of significant podocyte toxicity from STZ, we feel this is unlikely for several reasons: 1) other models of diabetic nephropathy that arise spontaneously show similar decreases in podocyte number and density; 2) relatively low dose STZ as used in these experiments has not been associated with proximal tubule toxicity and the proximal tubule cells should be especially sensitive to STZ since, like the pancreatic β cell, it expresses high levels of the facilitative glucose transporter, GLUT2 which transports STZ into cells; 3) finally and most importantly, we demonstrated that no changes in podocyte density or apparent number occurred within 3 days after completion of a 5-day low dose STZ protocol in mice, which strongly suggests that STZ has no acute podocyte toxicity. These 3-day data are depicted in Figure 3 and the reviewer’s point is explicitly discussed in a new paragraph in the discussion. Since other readers may like to see the graphical data because of issue of possible STZ toxicity we would ask to retain Figure 3.

For the GLEPP1 portion of figure 1, it is difficult to demonstrate pictorially the reduction in GLEPP1 positive cells because of the extended cytoplasmic distribution of this antigen. Counting of GLEPP1 positive cells is quite arduous as it requires repeated re-focusing to insure against double counting. Given these difficulties and the concurrence of GLEPP1 and WT-1 positivity, both Wiggins’ group and our laboratory have focused on WT-1 counting in current studies. We therefore have eliminated the GLEPP1 immunohistochemistry from Fig. 1.

Responses to Dr. Amann

We also very much appreciate these constructive comments and criticisms. First, as senior author, I have to apologize for not editing our manuscript as carefully as I should have. We had initially submitted this manuscript for publication to another journal over 2½ years ago when Dr. Siu left the laboratory to continue his training. After others in the laboratory performed additional experiments during the intervening period, I updated the manuscript but clearly failed to edit the abstract and introduction carefully enough. I am well aware that during that interval Dr. Amann’s group published convincing data on podocyte loss in diabetic nephropathy, and indeed we discussed and cited this work in our original BMC submission. Nonetheless, there were the aforementioned errors in the abstract and introduction and our discussion of Dr. Amann’s work was too limited. We have now corrected the abstract and added additional discussion of both papers by Gross, et al. Our methods, as noted and discussed extensively, are those developed by the Wiggins’ group and published in 2 papers (Kim, et al., Kidney International, 2001 and Sanden, et al, JASN, 2003, cited in our manuscript). These methods were originally reported to determine podocyte number. However, since the data are derived from representative rather than serial sections, it is more accurate to refer to the derived numbers as “apparent” to underline this methodological difference.
The precision of this methodology is high and these data reflect similar podocyte loss as other methods so appear to be accurate. Nonetheless, we have guarded against overinterpretation by using the qualifying term.

Dr. Amann noted the potential problems with the use of WT-1 and GLEPP1 as podocyte markers. Our methods of podocyte counting relied on accurately identifying podocyte nuclei (WT-1) or cytoplasm (GLEPP1). The WT-1 staining tends to be more precise since it is difficult to distinguish the cytoplasmic extent of any single podocyte using these methods. For these studies we followed the methods developed by the Wiggins’ laboratory. As noted by these investigators, a potentially disadvantage of this method is that podocyte nuclei are not uniformly WT-1 positive under some pathologic circumstances, as noted by Dr. Amann and in the more recent report from the Wiggins’ laboratory (Sanden, et al., JASN, 2003). However, as was noted in that report, there are no data to indicate that podocytes in diabetic nephropathy are WT-1 negative. In addition, WT-1 is an excellent marker of the normal mature differentiated podocyte and even podocytes with effaced foot processes remain WT-1 positive. Hence, if a podocyte is WT-1 negative, then it is likely to be severely and perhaps terminally damaged. Thus, we believe that our data are consistent with true and quite early reductions in podocyte density in experimental diabetic nephropathy and at the least reflect severe podocyte damage. These points have been added to the Discussion.

Dr. Amann also suggested using nephrin immunohistochemistry since it has been reported that glomerular nephrin levels are reduced in diabetic nephropathy. While this is true it is not certain how demonstrating a reduction of nephrin positive cells would aid our examination other than by adding an additional podocyte marker that is not necessarily any more accurate than the 2 markers we have already utilized. The issue of nephrin loss in diabetic nephropathy is acknowledged in the Discussion. Finally, the reviewer noted that we had not specifically discussed the differences between mouse and rat data at 2 weeks of diabetes. We have now added such a comparison to our discussion.

It should be emphasized that the point of our submission is not that there is podocyte loss in animal models of diabetes. This has been reported already by Dr. Amann’s group. What we do show for the first time is that podocyte number and density (or at least the number and density of reasonably healthy podocytes as determined by WT-1 positivity) are reduced quite early in diabetes and that these changes can be virtually completely prevented by treatment with lipoic acid.

We appreciate Dr. Amann’s direction to the minor revisions. We have now made all of the suggested changes.

Thank you again for considering this revised manuscript.

Sincerely,

Frank C. Brosius