Author's response to reviews

Title: Assessment of renal function in mice with unilateral ureteral obstruction using 99mTc-MAG3 dynamic scintigraphy

Authors:

Mohammed N Tantawy (n.tantawy@Vanderbilt.Edu)
Rosie Jiang (rjiang4@jhmi.edu)
Feng Wang (feng.wang.1@Vanderbilt.Edu)
Keiko Takahashi (keiko.takahashi@vanderbilt.edu)
Todd E Peterson (todd.e.peterson@Vanderbilt.Edu)
Dana Zemel (dana.zemel@Vanderbilt.edu)
Chuan-Ming Hao (chuanming.hao@Vanderbilt.Edu)
Hiroki Fujita (hirofuji@gipc.akita-u.ac.jp)
Raymond C Harris (raymond.harris@Vanderbilt.Edu)
Christopher C Quarles (chad.quarles@Vanderbilt.Edu)
Takamune Takahashi (takamune.takahashi@vanderbilt.edu)

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Author's response to reviews: see over
September 6, 2012

Prof. Hayley Henderson
Executive Editor
The BioMed Central Editorial Team

Dear Prof. Henderson:

I am submitting a revised manuscript (Manuscript ID 1383657750748593), entitled "Assessment of renal function in mice with unilateral ureteral obstruction using $^{99m}$Tc-MAG3 dynamic scintigraphy", for publication consideration in BMC-Nephrology.

We submitted the revised manuscript on August 6, 2012. However, we realized that our responses to the reviewer #1 were inadequate. Therefore, we further amended the manuscript.

If possible, would you mind sending this latest manuscript to the reviewers?

We are terribly sorry to interrupt you. We deeply apologize to you for the inconvenience. We think the latest manuscript has addressed all the issues adequately and will be suitable for publication.

The manuscript has not been published previously and is not being considered concurrently by another publication. There is no financial interest in the subject matter or materials discussed in the article.

Thank you very much for your efforts on behalf of our work.

Sincerely yours,

Takamune Takahashi M.D., Ph.D.
Assistant Professor of Medicine
Division of Nephrology and Hypertension
Vanderbilt University Medical Center
S-3223, Medical Center North, Nashville, TN37232
Phone: (615) 343 4312, Fax: (615) 343 7156
E-mail:takamune.takahashi@vanderbilt.edu
Responses to reviewers:

Dear Reviewers,

We submitted the revised manuscript on August 6, 2012. However, we realized that our responses to the reviewer #1 were inadequate. Therefore, we further amended the manuscript as follows. Our responses to the reviewer #2 are same as the previous revision (submitted on August 6).

We are so sorry to interrupt you. We deeply apologize to you for the inconvenience.

Thank you so much for reviewing our manuscript!

Sincerely,

Takamune Takahashi M.D., Ph.D.

Reviewer 1

My main concern relates to their overinterpretation of what these data mean. Basically, glomerular and tubular function in the obstructed kidney quickly decline. There is nothing about perfusion or blood flow that can be derived at all. With the conclusions modified, this is important work. The authors need to be cautious about statements relating to what the TACs of MAG3 are actually measuring. MAG3 enters the urine from both glomerular filtration and tubular secretion, and is a reasonable surrogate for hippurate, traditionally used to determine effective renal plasma flow because it is nearly completely removed by both processes in a single pass (MAG3 being protein bound is less of both). The utility of MAG3 only comes about because of water extraction by functioning tubules, thereby concentrating the tracer above background to allow determination of an ROI. The subsequent decay is due to its excretion via normal routes. In instances where there is altered glomerular filtration and tubular function, there is impairment in the ability of the kidney to extract MAG3, which can bear no relation at all to renal plasma flow. Thus, the use of "renal function" as in the title is appropriate. But, thereafter the mention of "perfusion," "renal blood flow," "functional blood vessels," is ubiquitous and need to be changed.

In the results section discussing figure 4, the passage "indicating that 99mTc-MAG3 is not excreted in these kidneys," which is an accurate statement, is immediately followed by the inaccurate, "profound reduction in renal perfusion." The authors provide measurements around the MAG3 peak, including the time post injection, slope(s) and magnitude. Other than the time to peak, the other data are overinterpreted. The slope for the "linear" phase may approximately reflect renal plasma flow, but this has several underlying variables. That the derived data actually represent a true "MAG3 concentration" in the kidney is a tremendous stretch. The authors need to report graphically what they are measuring, which are the COI cpm over time, and that alone.

[Additional Revision]

We really agree with you in that our statements and conclusions are over interpretation. The rising phase of TACs indicates renal uptake (accumulation) of 99mTc-MAG3. Although the linear rising pattern suggests that MAG3 uptake is relative to renal perfusion, this phase indicates both renal perfusion and tubular uptake, but not limited to renal perfusion. In this
context, we truly agree with you in that we need to be cautious about statements relating to what the TACs of MAG3 are actually measuring. Therefore, we corrected the statements and conclusions about this point and also amended the supplemental Figure 1. Our previous responses were also attached below.

[Previous Revision]

Thank you so much for your comments and suggestions. We agree with the reviewer in that the manuscript needs to further explain about what the TACs of MAG3 are actually measuring. Based on your comments, we revised the manuscript as follows.

MAG3 is known to be relatively high (75-90%) protein binding and its renal and plasma clearance is lower than hippurate clearance (49-67% of hippurate clearance). Also, it is known that glomerular clearance of MAG3 (11%) is lower than that of hippurate (27%). Therefore, it is thought that accurate estimation of effective renal plasma flow (ERPF) is difficult with MAG3 clearance, while clearance of radioiodinated orthoiodohippuric acid (OIH) is close to that of the reference standard paraaminohippuric acid (PAH). [Note: Radio-labeled OIH is well suited for ERPF measurement. However, high radiation absorbed dose, poor imaging quality because of the high energy of the gamma rays, and cost of the radiotracer made it difficult to use OIH in routine clinical practice. Radio-labeled OIH is no longer available in the US.]

Thus, MAG3 clearance is not suitable for ERPF estimation. Nevertheless, scintigraphic assessment of renal MAG3 kinetics is thought to provide a useful assay for evaluating renal function (perfusion and tubular function). The “dynamic” planar scan (frame/sec) enables “real-time” assessment of renal MAG3 kinetics. The influx of MAG3 is thought to reflect renal perfusion, while subsequent decay phase indicates tubular function (excretion), as described in the introduction. The parametric analysis of TACs, which is described in the manuscript, is widely used for assessing renal perfusion, while the decay rate [e.g. the time from peak to half peak activity (T1/2), the peak/10min ratio] is utilized as a parameter of tubular function (excretion), as described in the manuscript. The slope of linear uptake (or the time from influx to half peak activity) is generally used as a parameter of renal blood flow. Indeed, the changes in the slope of linear phase in UUO kidney are consistent with the results of previous reports about renal artery blood flow measurements (using a flow probe) of UUO kidneys, as described in the discussion. Also, a linear correlation between the fractional arterial flow parameters and renal MAG3 uptake has been shown in human obstructed kidneys (World J Urol 29(1):109-114, 2010). Thus, TACs of dynamic planar scan enables us to assess renal perfusion without measuring MAG3 clearance. [Notes: 1. MAG3 excretion and concentration are not required to produce renal radio-signals. 2. We are not assessing renal plasma flow by MAG3 clearance.]

Parametric analysis of dynamic MAG3 renal scintigraphy provides assessment of renal functions, renal perfusion and tubular function (excretion). Its utility is not limited to assessment of renal perfusion. However, tubular excretion rate (decay of MAG3) can not be assessed in UUO kidney due to lack of urine outflow. [For UUO kidney, what we can assess with dynamic MAG3 renal scintigraphy is renal perfusion parameters.] Also, as the reviewer pointed out, MAG3 excretion to urine (renal pelvis) is largely reduced in UUO kidney due to impaired tubular function and reduced glomerular filtration. Therefore, the TACs in UUO kidneys, from influx to peak, are thought to mostly indicate renal perfusion in parenchyma.

These are the reasons why we used the words, “perfusion”, “renal blood flow”, and “functional blood vessels”. However, we fully agree with the reviewer in that further explanation is required about this. Therefore, we explained these points in more detail in the manuscript and abstract.
(“highlighted in red”) with a graphic figure (additional file 1) and static Mag3 imaging data (Fig.6) so that readers can know what the TACs of MAG3 are indicating. [Notes: 1. The spatial resolution of dynamic MAG3 scintigraphy is highly limited due to short scan time (frame/sec). Therefore, renal MAG3 distribution can not be assessed by this mode of imaging. In contrast, static scan (minutes order scan) provides higher resolution imaging data, providing spatial information, and the data indicates overall kinetics of MAG3 during the scan period. 2. We think there is blood flow in UUO kidneys because the urine pool in renal pelvis progressively increases in these kidneys (Fig.1).]

Reviewer 2

Thank you so much for your comments and suggestions. According to your instructions, we revised the manuscript as follows.

1) Page numbers should be placed at bottom of each page.

We corrected the page numbering.

2) In reference 9, in press manuscript is difficult.

This reference has been published. Therefore, we included the publication information (year, volume, issue, pages) of this reference.

3) Journal titles in References are not abbreviated in accordance with the style of index medicus (example 13,14, 24, 28).

We carefully checked the references and corrected journal titles in accordance with the style of index medicus (ref. 5, 13,14, 24, 28).

4) The histological pictures need explain of the details (T1W and T2W in Figure 1).

We truly agree with the reviewer. We explained the findings in more detail by labeling the pictures (please see Figure 1 and its legend).