Journal of clinical and Translational Research
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Special issue article <Negative results>

# Administration of DDAVP did not improve the pharmacokinetics of FVIII concentrate in a clinically significant manner

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Handling editor:
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Department of Experimental Surgery, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands

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1st Editorial Decision
Date: 5 Dec, 2017

Subject: A decision has been made on JCTRes-D-17-00020
Ref.: Ms. No. JCTRes-D-17-00020
The effect of a DDAVP-induced rise in pre-infusion von Willebrand Factor levels on the pharmacokinetics of infused factor VIII in hemophilia A patients
Journal of Clinical and Translational Research

Dear authors,

An expert in the field has now commented on your paper. You will see that the reviewer is advising that you revise your manuscript. If you are prepared to undertake the work required, I would be pleased to reconsider my decision.

For your guidance, reviewers' comments are appended below.

If you decide to revise the work, please submit a list of changes or a rebuttal against each point which is being raised when you resubmit your work.

Your revision is due by Jan 04, 2018.

To submit a revision, go to http://jctres.edmgr.com/ and log in as an Author. You will see a menu item called Submission Needing Revision. You will find your submission record there.

Yours sincerely,

Michal Heger
Editor-in-Chief
Journal of Clinical and Translational Research

Editor's comments:

In preparing a revision I kindly ask you to pay particular attention to the fact that the FVIII preparation that was used already contains vWF, explaining why pharmacological doubling plasma VWF levels in patients prior to FVIII (concentrate) infusion has little effect on pharmacokinetics. Please note the differences between recombinant FVIII (does not contain vWF) and FVIII concentrate (used in this study- contains vWF) and highlight this in the manuscript to make it clear to readers. Accordingly, we also ask for utmost clarity in title and paper, underscoring that this strategy is not viable because the FVIII concentrate already contains vWF.

Thank you and kindest regards,

Michal.

Reviewers' comments:

Reviewer #1: Summary
The authors have tried to improve FVIII pharmacokinetics by pre-stimulation of VWF release through DDAVP. Hypothetically, in vivo formation of FVIII/VWF complexes would extend FVIII half-life, mainly because of VWF's known role as protective carrier protein. Naturally, this would only work for FVIII products that are devoid of VWF (i.e. recombinant FVIII products).
The approach is logical to follow from the beginning and interesting, although perhaps not very practical in a clinical setting (which is not of major importance for my evaluation). The authors report a convincing negative result and conclude that pharmacokinetics of infused factor VIII did not change in a meaningful way by increasing plasma VWF levels. It appears that all the work was carefully done.
However, I find it very surprising that a preparation of FVIII was infused that already contains VWF (>2x the molar amount of FVIII in the preparation). The VWF in this affinity purified preparation is complexed to FVIII (it was co-purified) and acts as the carrier protein, stabilizing FVIII (as is well known). Hence, to my opinion the main conclusion that "a DDAVP-induced rise in pre-infusion von Willebrand Factor levels does not alter the pharmacokinetics of infused factor VIII in hemophilia A patients" is incorrect. In fact, "a DDAVP-induced rise in pre-infusion von Willebrand Factor levels does not alter the pharmacokinetics of infused factor VIII/VWF complex in hemophilia A patients", which is not so surprising.

Major points
The title does not clearly reflect the main negative finding. The sentence in the abstract "Administration of DDAVP did not improve the pharmacokinetics of FVIII in a clinically significant manner." reads a lot more accurate, with addition of the word concentrate.
Similarly, throughout the paper "FVIII half-life" is discussed. Really, we have been looking at "FVIII

concentrate half-life (which already contains VWF)". This requires revision to accurately reflect what was investigated.

In the discussion, the authors compare their work to references 13 and 20. However, in reference 13 r-VIII SQ was used. This recombinant FVIII that does not contain VWF, but can bind to it (http://onlinelibrary.wiley.com/doi/10.1111/j.1432-1033.1995.tb20776.x/pdf), which would allow the protective carrier function of VWF after DDAVP treatment. Reference 30 uses FVIII concentrate, which contains VWF. Hence, the sentence "At that time, FVIII concentrates contained vWF in a 1:1 molar basis compared to FVIII." only appears to apply to reference 30, but not to 13. As this information is key to understanding the differences between papers, it needs to be revised.

Discussion: "the FVIII concentrate used in this study contains residual amounts of vWF" this is a very remarkable statement. This FVIII concentrate contains twice the molar amount of VWF than it contains FVIII. It was purified together so most probably in complex.

Minor points
-Figure 1 does not contribute much to the paper.

********Authors' rebuttal********

Editor's comments:
In preparing a revision I kindly ask you to pay particular attention to the fact that the FVIII preparation that was used already contains vWF, explaining why pharmacological doubling plasma VWF levels in patients prior to FVIII (concentrate) infusion has little effect on pharmacokinetics. Please note the differences between recombinant FVIII (does not contain vWF) and FVIII concentrate (used in this study- contains vWF) and highlight this in the manuscript to make it clear to readers. Accordingly, we also ask for utmost clarity in title and paper, underscoring that this strategy is not viable because the FVIII concentrate already contains vWF.

**Answer to the editor:**
**We are grateful for your commentary and suggestions, which we have addressed to the fullest extent.**
**We apologize for the unclarity regarding the type of FVIII concentrate we used in our manuscript. CLB-factor VIII-M is a plasma product which contains 100 nanograms vWF for every Unit of FVIII. The product specification states that CLB-factor VIII-M does not contain functional vWF. In general, the stoichiometry of FVIII/vWF is 1:50. This means that 50 monomers of vWF are needed for the binding of every molecule of FVIII.**
**As we described on page 8, CLB-factor VIII-M contains 100 IU/mL FVIII after dilution. As the concentration of FVIII is 100 Units/mL, the 100 nanograms of vWF/Unit in solvation equals 10 ug/mL VWF. The molar ratio's therefore are: 1 IU FVIII/mL is 0.4 nM and therefore 100 IU FVIII/mL is 40 nM FVIII; 10 ug/mL VWF equals 1 IU/mL and this is 50 nM.**
**As the ratio for stoichiometry of FVIII to bind vWF is 1:50, the 40nM FVIII/50nM vWF ratio is not sufficient for vWF to be a functional carrier of FVIII. The amount of vWF present in CLB-factor VIII-M is not large enough to bind FVIII in complexes. Therefore, we do think that our strategy is viable.**

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The authors have tried to improve FVIII pharmacokinetics by pre-stimulation of VWF release through DDAVP.

Hypothetically, in vivo formation of FVIII/VWF complexes would extend FVIII half-life, mainly because of VWF's known role as protective carrier protein. Naturally, this would only work for FVIII products that are devoid of VWF (i.e. recombinant FVIII products).
The approach is logical to follow from the beginning and interesting, although perhaps not very practical in a clinical setting (which is not of major importance for my evaluation). The authors report a convincing negative result and conclude that pharmacokinetics of infused factor VIII did not change in a meaningful way by increasing plasma VWF levels. It appears that all the work was carefully done. However, I find it very surprising that a preparation of FVIII was infused that already contains VWF (>2x the molar amount of FVIII in the preparation). The VWF in this affinity purified preparation is complexed to FVIII (it was co-purified) and acts as the carrier protein, stabilizing FVIII (as is well known). Hence, to my opinion the main conclusion that "a DDAVP-induced rise in pre-infusion von Willebrand Factor levels does not alter the pharmacokinetics of infused factor VIII in hemophilia A patients" is incorrect. In fact, "a DDAVP-induced rise in pre-infusion von Willebrand Factor levels does not alter the pharmacokinetics of infused factor VIII/VWF complex in hemophilia A patients", which is not so surprising.

**Answer to the reviewer:**
**We thank the reviewer for stressing the importance of the message of our manuscript and for the highly valuable suggestions.**
**We apologize for the unclarity regarding the type of FVIII concentrate we used in our manuscript.**
**CLB-factor VIII-M is a plasma product which contains 100 nanograms vWF for every Unit of FVIII. The product specification states that CLB-factor VIII-M does not contain functional vWF. In general, the stoichiometry of FVIII/vWF is 1:50. This means that 50 monomers of vWF are needed for the binding of every molecule of FVIII.**
**As we described on page 8, CLB-factor VIII-M contains 100 IU/mL FVIII after dilution. As the concentration of FVIII is 100 Units/mL, the 100 nanograms of vWF/Unit in solvation equals 10 ug/mL VWF. The molar ratio's therefore are: 1 IU FVIII/mL is 0.4 nM and 100 IU/mL is 40 nM FVIII; 10 ug/mL VWF equals 1 IU/mL and this is 50 nM.**
**As the ratio for stoichiometry of FVIII to bind vWF is 1:50, the 40nM FVIII/50nM vWF ratio is not sufficient for vWF to be in complex with FVIII. The amount of vWF present in CLB-factor VIII-M is not large enough to bind FVIII in complexes. Therefore, we do think that our strategy is viable. We added this information to page 8.**
Major points
The title does not clearly reflect the main negative finding. The sentence in the abstract "Administration of DDAVP did not improve the pharmacokinetics of FVIII in a clinically significant manner." reads a lot more accurate, with addition of the word concentrate.
**Thank you very much for your suggestion. We changed the title into: "Administration of DDAVP did not improve the pharmacokinetics of FVIII concentrate in a clinically significant manner"**
Similarly, throughout the paper "FVIII half-life" is discussed. Really, we have been looking at "FVIII concentrate half-life (which already contains VWF)". This requires revision to accurately reflect what was investigated.
**Thank you for your request for specification of FVIII, we changed the use of the word FVIII half-life into FVIII concentrate half-life throughout the manuscript.**
In the discussion, the authors compare their work to references 13 and 20. However, in reference 13 r-VIII SQ was used. This recombinant FVIII that does not contain VWF, but can bind to it (http://onlinelibrary.wiley.com/doi/10.1111/j.1432-1033.1995.tb20776.x/pdf), which would allow the protective carrier function of VWF after DDAVP treatment. Reference 30 uses FVIII concentrate, which contains VWF. Hence, the sentence "At that time, FVIII concentrates contained vWF in a 1:1 molar basis compared to FVIII." only appears to apply to reference 30, but not to 13. As this information is key to understanding the differences between papers, it needs to be revised.
**We are grateful for your critical reading of the manuscript.**
**We understand the confusion and deleted the sentence as we think the information is irrelevant after the explanation in the methods section after your valuable first question.**

Discussion: "the FVIII concentrate used in this study contains residual amounts of vWF" this is a very remarkable statement. This FVIII concentrate contains twice the molar amount of VWF than it contains FVIII. It was purified together so most probably in complex.
**Thank you for your highly valuable remark. However, we hope that our calculation in response to your major comment above clarifies that the amount of vWF was residual, taking the stoichiometry of FVIII/vWF into account.**

Minor points
-Figure 1 does not contribute much to the paper.
**Thank you for sharing this thought. We believe that Figure 1 does give some detailed information that is necessary to provide a theoretical framework behind the working mechanism of DDAVP and therefore we would like to leave it up to the editor to decide whether or not he wants to include the Figure.**

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2nd editorial decision

Date: 20 Dec, 2017

Subject: A decision has been made on JCTRes-D-17-00020R1
Ref.: Ms. No. JCTRes-D-17-00020R1
Administration of DDAVP did not improve the pharmacokinetics of FVIII concentrate in a clinically significant manner
Journal of Clinical and Translational Research

Dear author(s),

Reviewers have submitted their critical appraisal of your paper. The reviewers' comments are appended below. Based on their comments and evaluation by the editorial board, your work was FOUND SUITABLE FOR PUBLICATION AFTER MINOR REVISION.

If you decide to revise the work, please itemize the reviewers' comments and provide a point-by-point response to every comment. An exemplary rebuttal letter can be found on at http://www.jctres.com/en/author-guidelines/ under "Manuscript preparation." Also, please use the track changes function in the original document so that the reviewers can easily verify your responses.

Your revision is due by Jan 19, 2018.

To submit a revision, go to http://jctres.edmgr.com/ and log in as an Author. You will see a menu item call Submission Needing Revision. You will find your submission record there.

Yours sincerely,

Michal Heger
Editor-in-Chief
Journal of Clinical and Translational Research

Reviewers' comments:

Reviewer #1: The authors have amended the manuscript as requested for the major part. I am a bit

surprised by the response to my most important critisism; by providing a new set of calculations, the authors conclude that there is little/no VWF in the preparation, but certainly have not convinced me of this. My comment was/is about the stochiometry of FVIII and VWF in the preparation. Even based on the data provided in the revision, it is still obvious that FVIII and VWF are present in the FVIII concentrate in a 1:1 molar ratio (outlined in detailed below for clarification). This is not a problem, but the calculations in the revision obscure this, which is both unnecesary and confusing. Later on it is concluded that it is insufficient for complex formation. The rationale for this statement is unclear and not supported by (referenced) biochemical evidence.

In the original version of the manuscript: "After reconstitution, this solvent detergent (SD) treated FVIII concentrate contains 100 IU/ml (40nM) FVIII and 1.74 IU/ml (87 nM) vWF." Was this 87 nM figure wrong? Why is it eliminated from the updated manuscript? Please express the molar concentration of VWF in the preparation as you did for FVIII. This is the only thing I will request. In the new calculations 50 nM is found. I am fine with this figure, too, but not with the rationale that this is insuffucient for complex formation. I suggest to change the complete relevant section of the methods section to " "After reconstitution, this solvent detergent (SD) treated FVIII concentrate contains 100 IU/ml (40nM) FVIII and (50 nM) vWF." and remove all the sentences on whether or not this enables complex formation or whether there is a lot or a little VWF in FVIII concentrate.

-------detailed information-------
In the revision, information on the levels of VWF in the FVIII preparation were given, using Units, nanograms and molarity interspersely. This is confusing. I will run through the calculations and potential errors step by step below.

Current version: "After reconstitution, this solvent detergent (SD) treated FVIII concentrate contains 100 IU/mL (40 nM) FVIII, which contains 100 ng VWF per Unit FVIII."
---> Let's calculate this. 100 (IU FVIII) x100ng (VWF)/mL = 10 ug/mL VWF. This equals 40 nM (assuming a molecular weight of 250 kDa). This means the molar ratio FVIII:VWF is 1:1.

"The product specification states that CLB-factor VIII-M does not contain functional vWF".
---> Does this statement relate in any way to it's function as carrier of FVIII? If not, it is not very relevant.

"In general, the stoichiometry of FVIII/vWF is 1:50. This means that 50 vWF molecules are needed for the binding of every molecule of FVIII."
---> I am not sure I agree here, how do the authors come to this conclusion? VWF is a multimeric protein. Was this taken into account? The molecular binding interactions between FVIII and VWF are well-known; they form 1:1 complexes. How would one FVIII molecule (280 kDa) be capable of binding 50 VWF molecules (250 kDa each)? Even if there were 50:1 VWF molecules per FVIII molecule in the circulation of a healthy person, by no means does this indicate that a 1:1 ratio does not provide protection against FVIII clearance. There is simply not much FVIII in normal plasma, compared to the amount of VWF (excess). This does not mean at all that FVIII requires 50x more VWF to form complexes.

"CLB-factor VIII-M contains 100 IU/mL FVIII after dilution. As the concentration of FVIII is 100 Units/mL, the 100 ng of vWF/Unit in solvation equals 10 μg/mL VWF."
---> I agree (by the way, what is solvation?). Let's take this further. Assuming that FVIII has a molecular weight of 280 kDa, a 40 nM FVIII concentration equals 11.2 ug/mL. Do the authors agree

that this is nearly the same? So the message in the paper that FVIII concentrate contains a lot of FVIII and little to no VWF to my opinion is inaccurate. Both on a molar and a mass ratio FVIII and VWF are present in near-equal amounts, which helps to explain the findings.

"The molar ratios therefore are: 1 IU FVIII/mL is 0.4 nM and 100 IU/mL is 40 nM FVIII"
It was stated before that 100 IU/mL equals 40 nM FVIII.

"....10 μg/mL VWF equals 1 IU/mL and this is 50 nM."
---> this is an unclear sentence. In the previous version the concentration of VWF was 1.74 IU/ml, but lets assume that was an error. I end up with 40 nM, but 50 nM is also fine to me. So the authors agree that there is at least as much VWF in FVIII concentrate as there is FVIII? This needs to be stated clearly.

"As the ratio for stoichiometry of FVIII to bind vWF is 1:50.."
---> This is the part that troubles me the most. Please reconsider this (unreferenced) assumption, because it appears to be based on the ratio between VWF and FVIII that is present in plasma (where much more free VWF is present than VWF). It is dangerous to assume that this ratio is required for complex formation. The available biochemical data has clearly demonstrated that FVIII and VWF can form 1:1 complexes through interactions between FVIII's A3 / C-domains and the D'-D3 sequence in VWF. VWF multimerization is not prerequisite for complex formation.

"....the 40 nM FVIII/50 nM vWF ratio is not sufficient for vWF to be in complex with FVIII. The amount of vWF present in CLBfactor VIII-M is not large enough to bind FVIII in complexes"
---> So the authors do agree that on a molar basis, there is at least an equal amount of VWF molecules present in the FVIII concentrate. I recommend to clarify this as best as one can. For the rest, I do not agree with this rationale at all. Since it is not needed for the paper, I suggest to remove it.

********Authors' rebuttal********

Reviewers' comments:

**Reviewer #1: The authors have amended the manuscript as requested for the major part. I am a bit surprised by the response to my most important critisism; by providing a new set of calculations, the authors conclude that there is little/no VWF in the preparation, but certainly have not convinced me of this. My comment was/is about the stochiometry of FVIII and VWF in the preparation. Even based on the data provided in the revision, it is still obvious that FVIII and VWF are present in the FVIII concentrate in a 1:1 molar ratio (outlined in detailed below for clarification). This is not a problem, but the calculations in the revision obscure this, which is both unnecesary and confusing. Later on it is concluded that it is insufficient for complex formation. The rationale for this statement is unclear and not supported by (referenced) biochemical evidence.**

Answer to the reviewer:

*We kindly thank the reviewer for outlining this and we agree that the molar ratio of FVIII and VWF is 1:1. We certainly did not intend to obscure this and therefore we have rewritten this part of the manuscript to clearly state that the molar ratio of FVIII and VWF is 1:1 in the preparation used. Please find our explanation regarding the rationale below in the answers to the detailed comments.*

**In the original version of the manuscript: "After reconstitution, this solvent detergent (SD) treated FVIII concentrate contains 100 IU/ml (40nM) FVIII and 1.74 IU/ml (87 nM) VWF." Was this 87 nM figure wrong? Why is it eliminated from the updated manuscript? Please express the molar concentration of VWF in the preparation as you did for FVIII. This is the only thing I will request. In the new calculations 50 nM is found. I am fine with this figure, too, but not with the rationale that this is insuffucient for complex formation. I suggest to change the complete relevant section of the methods section to " "After reconstitution, this solvent detergent (SD) treated FVIII concentrate contains 100 IU/ml (40nM) FVIII and (50 nM) VWF." and remove all the sentences on whether or not this enables complex formation or whether there is a lot or a little VWF in FVIII concentrate.**

Answer to the reviewer:

*We sincerely apologize for the confusion regarding the number 87. We changed the complete relevant section of the methods section as suggested by the reviewer. In the Discussion we present the result and discuss the findings of studies reporting on the stoichiometry of binding of FVIII to VWF.*

-------detailed information-------

**In the revision, information on the levels of VWF in the FVIII preparation were given, using Units, nanograms and molarity interspersely. This is confusing. I will run through the calculations and potential errors step by step below.**

**Current version: "After reconstitution, this solvent detergent (SD) treated FVIII concentrate contains 100 IU/mL (40 nM) FVIII, which contains 100 ng VWF per Unit FVIII."**

**---> Let's calculate this. 100 (IU FVIII) x100ng (VWF)/mL = 10 ug/mL VWF. This equals 40 nM (assuming a molecular weight of 250 kDa). This means the molar ratio FVIII:VWF is 1:1.**

Answer to the reviewer:

*This step is indeed correct, the molar ratio is 1:1.*

**"The product specification states that CLB-factor VIII-M does not contain functional VWF".**

**---> Does this statement relate in any way to it's function as carrier of FVIII? If not, it is not very relevant.**

Answer to the reviewer:

*This statement has been included by the manufacturer. We agree with the reviewer that it is not very relevant. We do not know the reason for including this statement in the product specification. We suspect that it has been included to show that CLB-factor VIII-M should not be used for treatment of VWD. The functionality of the low amounts of VWF in the preparation has not been investigated therefore it is not possible to make a statement of the functionality of VWF in CLB-factor VIII-M. Based on these considerations we have decided not to include this statement in the manuscript and only refer to a previous clinical study in which the properties of CLB-factor VIII-M were described* (1).

**"In general, the stoichiometry of FVIII/VWF is 1:50. This means that 50 VWF molecules are needed for the binding of every molecule of FVIII."**

**---> I am not sure I agree here, how do the authors come to this conclusion? VWF is a multimeric protein. Was this taken into account? The molecular binding interactions between FVIII and VWF are well-known; they form 1:1 complexes. How would one FVIII molecule (280 kDa) be capable of binding 50 VWF molecules (250 kDa each)?**

Answer to the reviewer:

*We thank the reviewer for her/his interesting comment.*

*Early studies on the binding of FVIII to VWF have provided evidence for multiple classes of FVIII-binding sites on VWF* (2). *This study concluded that "The high-affinity binding (Kd = 2.1 x 10(-10) M) was restricted to only 1-2% of the VWF subunits. Competition studies with monoclonal antibodies with known epitopes demonstrated that the Factor VIII sequence Lys1673-Arg1689 is involved in the high-affinity interaction with VWF." This observation is consistent with the molar ratio of FVIII/VWF of 1:50 in the circulation. It is currently not known why only 1-2% of the VWF subunits can bind FVIII with high affinity.*

*Using velocity sedimentation analysis Lollar and Parker provided evidence that one factor VIII-binding site is present per VWF monomer, these results indicate that all factor VIII-binding sites are accessible in the VWF multimer* (3).

*The differences observed between the two studies show that the stoichiometry of binding may depend on the technology used to assess binding.*

*We suspect (but we cannot prove) that the differences observed are due the presence of multiple classes of binding sites for FVIII on VWF. Low affinity binding of FVIII may occur to all VWF subunits yielding a stoichiometry of 1:1 whereas high affinity binding of FVIII may occur to a subset of VWF subunits (for instance the terminal VWF subunits in a VWF multimer) with a stoichiometry of binding of 1:50.*

*Competition studies with monoclonal antibodies with known epitopes demonstrated that the Factor VIII sequence Lys1673-Arg1689 is involved in the high-affinity interaction with VWF* (2). *Furthermore sulfation of Tyr1680 has been shown to be a crucial for high affinity binding to VWF* (4). *The physiological importance of this high affinity binding site on FVIII for VWF is suggested by the low FVIII levels observed in patients carrying a Tyr1680 to Phe substitution (median baseline FVIII:C in 20 patients in INSIGHT study was 5 IU/dL (IQR 2-31))* (5).

*We have adapted the manuscript and included references to previous studies that reported on the stoichiometry of binding of FVIII to VWF. We hope we have been able to discuss the still unresolved issue of the stoichiometry of binding of FVIII to VWF in a balanced manner.*

**Even if there were 50:1 VWF molecules per FVIII molecule in the circulation of a healthy person, by no means does this indicate that a 1:1 ratio does not provide protection against FVIII clearance. There is simply not much FVIII in normal plasma, compared to the amount of VWF (excess). This does not mean at all that FVIII requires 50x more VWF to form complexes.**

Answer to the reviewer:

*We agree with the reviewer and provide more explanation in the discussion.*

*Off note, in response to this question, we would like to refer to Borchiellini A et al* (6). *They showed that "although the typical plasma concentration of FVIII is 100–250ng mL−1 (approximately 1 nM),*

*the plasma concentration of VWF is approximately 8 μg mL−1 (approximately 50 nM).” Thus there is a 30–50 m excess of VWF to FVIII in normal circulation, such that not all VWF multimers contain FVIII.*

**"CLB-factor VIII-M contains 100 IU/mL FVIII after dilution. As the concentration of FVIII is 100 Units/mL, the 100 ng of VWF/Unit in solvation equals 10 µg/mL VWF."**

**---> I agree (by the way, what is solvation?).**

Answer to the reviewer:

*The correct word is solution, our apologies. This means that it is dissolved in sterile water.*

**Let's take this further. Assuming that FVIII has a molecular weight of 280 kDa, a 40 nM FVIII concentration equals 11.2 ug/mL. Do the authors agree that this is nearly the same? So the message in the paper that FVIII concentrate contains a lot of FVIII and little to no VWF to my opinion is inaccurate. Both on a molar and a mass ratio FVIII and VWF are present in near-equal amounts, which helps to explain the findings.**

Answer to the reviewer:

*It is true that the amount of FVIII and VWF in grams is about the same in the concentrate, and also that the molar ratio is 1:1. We have further emphasized this in the revised manuscript.*

**"The molar ratios therefore are: 1 IU FVIII/mL is 0.4 nM and 100 IU/mL is 40 nM FVIII"**

**It was stated before that 100 IU/mL equals 40 nM FVIII.**

**"....10 µg/mL VWF equals 1 IU/mL and this is 50 nM."**

**---> this is an unclear sentence. In the previous version the concentration of VWF was 1.74 IU/ml, but lets assume that was an error. I end up with 40 nM, but 50 nM is also fine to me. So the authors agree that there is at least as much VWF in FVIII concentrate as there is FVIII? This needs to be stated clearly.**

Answer to the reviewer:

*We indeed agree.*

**"As the ratio for stoichiometry of FVIII to bind VWF is 1:50.."**

**---> This is the part that troubles me the most. Please reconsider this (unreferenced) assumption, because it appears to be based on the ratio between VWF and FVIII that is present in plasma (where much more free VWF is present than VWF). It is dangerous to assume that this ratio is required for complex formation. The available biochemical data has clearly demonstrated that FVIII and VWF can form 1:1 complexes through interactions between FVIII's A3 / C-domains and the D'-D3 sequence in VWF. VWF multimerization is not prerequisite for complex formation.**

**"....the 40 nM FVIII/50 nM VWF ratio is not sufficient for VWF to be in complex with FVIII. The amount of VWF present in CLBfactor VIII-M is not large enough to bind FVIII in complexes"**

**---> So the authors do agree that on a molar basis, there is at least an equal amount of VWF molecules present in the FVIII concentrate. I recommend to clarify this as best as one can. For the rest, I do not agree with this rationale at all. Since it is not needed for the paper, I suggest to remove it.**

Answer to the reviewer:

*We have revised our manuscript and discuss the currently available data on the stoichiometry of the FVIII/VWF complex in the Discussion* (2,3)*. Based on the availability data we believe that high affinity binding of FVIII to VWF occurs with a stoichiometry of 1 in 50. For low affinity binding of FVIII to VWF as revealed by sedimentation a stoichiometry of 1 to has 1 been reported. Based on the low levels of FVIII in patients carrying a Tyr1680 to Phe mutation which disrupts high affinity binding to VWF we feel that the high affinity binding of FVIII to VWF (with a stoichiometry of 1 to 50) is a major determinant for circulating FVIII levels.*

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3rd editorial decision

Date: 23 Jan, 2018

Subject: A decision has been made on JCTRes-D-17-00020R2
Ref.: Ms. No. JCTRes-D-17-00020R2
Administration of DDAVP did not improve the pharmacokinetics of FVIII concentrate in a clinically significant manner
Journal of Clinical and Translational Research

Dear authors,

I am pleased to inform you that your manuscript has been accepted for publication in the Journal of Clinical and Translational Research.

If you can, please take a look at the reviewer's last comments, on which the decision is not predicated but may help you with the final version of your paper.

You will receive the proofs of your article shortly, which we kindly ask you to thoroughly review for any errors.

Thank you for submitting your work to JCTR.

Kindest regards,

Michal Heger
Editor-in-Chief
Journal of Clinical and Translational Research

Comments from the editors and reviewers:

Reviewer #1: Thank you for making these last amendments. Especially for the point-by-point discussion.
I still feel that some sentences related to my most important critique conflict with each other. This will

confuse the reader, but I will not ask for any more changes.

Example:
"the FVIII concentrate used in this study contains residual amounts of VWF which might partially obscure the effect of the DDAVP induced rise of VWF."
versus
"In the product used in this study, the molar ratio of FVIII/VWF was 1:1."

**References:**

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