Author’s response to reviews

Title: ROLE OF CATHEPSIN S IN PERIODONTAL WOUND HEALING - AN IN VITRO STUDY ON HUMAN PDL CELLS

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Version: 2 Date: 23 Feb 2018

Author’s response to reviews:

We are very grateful to the reviewers for their valuable time they spent on reading our manuscript and the constructive comments. We have addressed all issues and made great efforts to improve our manuscript as recommended. Additional experiments were performed and we hope that the revised version of our manuscript is now acceptable for publication in BMC Oral Health.
Technical Comments

Funding not included

Our response:

We have added the following information on our funding sources to the Declaration section (page 12; lines 1-3):

“This study was supported by the Medical Faculty of the University of Bonn, the University of Sydney, the German Orthodontic Society (DGKFO) and the German Research Foundation (DFG, ME 4798/1-1).”

Reply to reviewer #1 (Antonella Polimeni)

1. Concern of the reviewer:

Now the paper could be considered acceptable for publication.

Our response:

We would like to thank the reviewer for her positive comment on our manuscript and her valuable time.

Reply to reviewer #2 (Richard J. Miron)

1. Concern of the reviewer:

The authors have revised the manuscript accordingly. This is an excellent article from an experienced research group and merits publication in its present format.

Our response:

We are also very grateful the reviewer for reading our manuscript and making this very positive comment.

Reply to reviewer #3 (Reinhard Gruber)

1. Concern of the reviewer:

The authors show that CatS is a mitogen that also supports migration of periodontal fibroblasts. Even though the data are preliminary, the findings are new and of potential clinical value.
Our response:

We would like to thank the reviewer for his valuable time and the very detailed and constructive comments, which helped us to improve our manuscript. As recommended, we have performed additional experiments and added new data to the manuscript. In our previous study Fusobacterium nucleatum stimulated the synthesis of cathepsin S (CTSS). Moreover, in the experiments of the present manuscript, CTSS resulted in an enhanced in vitro wound closure. As suggested by the reviewer, we therefore sought to clarify in additional experiments whether periodontopathogens such as F. nucleatum could accelerate the in vitro wound closure and, if so, if the stimulatory effect would involve cathepsins. By these experiments, we combine the knowledge of our previous and present studies, as recommended.

2. Concern of the reviewer:

What a pity that the authors have not yet published their finding that CatS is found in inflamed periodontal tissue.

Our response:

At the time when we submitted our manuscript the findings mentioned by the reviewer were not published yet. Therefore, we could not provide a reference. However, in the meantime, these results can be found in the following article:


We thank the reviewer for his comment and have added the reference to the manuscript (reference number 20; page 14; lines 25-28).

3. Concern of the reviewer:

This putative finding is maybe not surprising considering that CatS mediate the PgLPS induced IL-6 expression in splenic cells PMID: 28769800. This CatS activity required PAR-2 activation. This research could be inspiring to test FSLLRY-NH2, a PAR2 antagonist, in the study by Memmert et al.

Our response:
We thank the reviewer for pointing at this interesting study. We agree with the reviewer that it would be very interesting to examine if periodontopathogenic microorganisms could induce periodontal inflammation through CTSS and protease-activated receptor (PAR) 2 activation. We therefore performed a PubMed search on this exciting topic. Unfortunately, there is only very limited evidence on the relationship between F. nucleatum and PAR1/PAR2 activation (Chung WO et al., Immunol Lett. 2010 Jul 8;131(2):113-9). Nevertheless, we incubated wounded PDL cell monolayers with a specific PAR2 agonist (SLIGKV-NH2, abcam, 200 μM) and analyzed the wound closure. Wounded monolayers in the absence of this PAR2 agonist served as control. Our experiments revealed that PAR2 had no significant effect on the in vitro wound closure. Therefore, no further analyses regarding the potential involvement of cathepsins were performed in this experimental setup.

4. Concern of the reviewer:

Also cysteine protease inhibitor could be used to see if the mitogenic activity of CatS requires the proteolytic activity of the enzyme. Authors could also rule out that faster wound closure is a consequence of CatS-induced cell proliferation, maybe by irradiating the cell or by blocking cell cycle genes.

Our response:

As suggested by the reviewer, we ordered a cathepsin inhibitor (Z-FA-FMK, Santa Cruz, 50 μM) to further study the effects of cathepsins on PDL cells. The respective findings will be described and discussed further below. In the present manuscript, cell proliferation in the presence and absence of CTSS was assessed by real-time PCR for PCNA, a marker of cell proliferation, and by a cell proliferation XTT assay. Irradiation of cells would have been interesting but unfortunately not feasible in our laboratory. Since our data from both assays were very consistent, no further proliferation analyses were performed.

5. Concern of the reviewer:

Also, CatK could serve as a nice control for proliferation.

Our response:

We thank the reviewer for this ingesting comment. Cathepsin K (CTSK) is closely related to CTSS and increased in periodontitis (Garg G et al., Arch Oral Biol. 2009 Nov;54(11):1046-51). Therefore, inhibition of CTSK can prevent bone loss and the immune response during the progression of periodontitis, suggesting that CTSK might be a promising target for treating periodontitis (Hao L et al., J Periodontol 2015;86:972-83; Gruber R, Wien Med Wochenschr.
2015 Feb;165(3-4):48-53). On the other side, CTSK might also have antibacterial and anti-inflammatory actions, as reported in a chronic intestinal inflammation model (Sina C et al., Gut 2013;62:520-530). In addition, CTSS has the ability to degrade CTSK, indicating complex interactions between CTSS and CTSK (Barry ZT & Platt MO, J Biol Chem. 2012 Aug 10;287(33):27723-30). Taken together, we completely agree with the reviewer that further research should also focus on the complex role of CTSK in PDL cell proliferation, migration and wound closure.

We have added the following sentence to the Discussion section (pages 9-10; lines 27-2):

“Cathepsin K (CTSK), another member of the cathepsin family, has also been associated with periodontal diseases [37-39]. Interestingly, CTSS has the ability to degrade CTSK, indicating complex interactions between both cathepsins [40]. Further research should also focus on the role of CTSK in PDL cell proliferation, migration and wound closure.”

We have added the following references to the Reference list (reference numbers 37-40; page 16; lines 15-23):


6. Concern of the reviewer:

To support the hypothesis, important would be to expose the fibroblasts to a TLR stimulus and then measure proliferation and migration - with antisense CatS RNA or better a specific CatS inhibitor Z-FL-COCHO, or at least cysteine protease inhibitors.

Our response:

We appreciate the reviewer’s suggestion to expose cells to a toll-like receptor (TLR) agonist and, subsequently, to study the cell proliferation/migration/wound closure. We are very grateful for this comment, because these experiments help to link our recently published study with the experiments of the present manuscript. As recommended, we purchased a TLR2 agonist
(Pam3CSK4, 1μg/ml, Invivogen), which was then used to incubate the wounded cell monolayers. Wounded monolayers in the absence of the TLR2 agonist served as control. Interestingly, exposure of the monolayers to the TLR2 agonist resulted in a significantly accelerated wound closure. Afterwards, we sought to determine whether the stimulatory effect of the TLR2 agonist on the wound closure is mediated through cathepsins. Since the use of antisense technique in PDL cells would require comprehensive pre-studies and a specific cathepsin S inhibitor was not available within the limited time for revision (we contacted several companies such as Sigma, Calbiochem, Adooq Bioscience etc.), we decided to order and to use the cathepsin inhibitor Z-FA-FMK (Santa Cruz Biotechnology, 50 μM). TLR2 agonist-treated wounded monolayers were incubated or not with this cathepsin inhibitor for 24 h and, subsequently, the wound closure was analyzed. These experiments revealed that the stimulation of the in vitro wound healing by the TLR2 agonist was indeed dependent on cathepsins. This finding is of major importance, because it links the experiments of this study with our previous findings. Moreover, since we used a TLR2 agonist and an unspecific cathepsin inhibitor, this result may have an even broader significance for the understanding of periodontal diseases, because periodontitis is not only caused by a single bacterium or mediated by a single member of the cathepsin family.

We have added the following information to the Abstract (page 2; lines 10-12, 15-17, 21-22):

“Additionally, PDL cell monolayers were treated with a toll-like receptor 2 agonist in the presence and absence of a cathepsin inhibitor to examine if periodontal bacteria can alter wound closure via cathepsins.”

“Moreover, the toll-like receptor 2 agonist enhanced significantly the wound closure and this stimulatory effect was dependent on cathepsins.”

“In addition, cathepsins might be exploited by periodontal bacteria to regulate critical PDL cell functions.”

We have added the following information to the Introduction section (page 3; lines 6-7):

“… such as Fusobacterium nucleatum … through binding to special receptors such as toll-like receptor (TLR) 2, which can ultimately result in … [3].”

We have added the following information to the Methods section (pages 4-5; lines 25-27, 7-8):

“In a subset of experiments, PDL cells were incubated with a TLR2 agonist (1 μg/ml; Pam3CSK4; Invivogen, San Diego, CA, USA) in the presence and absence of a cathepsin inhibitor (50 μM; Z-FA-FMK; Santa Cruz Biotechnology, Dallas, TX, USA) for 24 h [25, 26]. “

“…the TLR2 agonist in combination with or without the cathepsin inhibitor for 24 h, as described above.”
We have added the following information to the Results section (page 8; lines 1-9):

“Involvement of cathepsins in TLR2 effects on wound closure

The aforementioned experiments demonstrated that CTSS accelerates wound closure, and our previous experiments had revealed that F. nucleatum, which is able to activate TLR2, upregulates CTSS [20]. Therefore, we next sought to examine whether a TLR2 agonist would enhance the wound closure and whether such a potential stimulatory effect would involve cathepsins. As shown in Figure 4A, incubation of PDL cell monolayers with a TLR2 agonist enhanced significantly the average wound closure by approximately 70 % over 24 h. However, when the TLR2 agonist-treated monolayers were simultaneously exposed to a cathepsin inhibitor, the enhanced wound closure was significantly reduced by 50 % (Fig. 4B).”

We have added the following information to the Discussion section (pages 8-9; lines 15-17, 16-26):

“Moreover, TLR2 activation also accelerates the PDL cell wound closure and this stimulatory effect depends on cathepsins, suggesting that cathepsins might be exploited by periodontal bacteria to regulate critical PDL cell functions.”

“Notably, exposure of the PDL cell monolayers to the TLR2 agonist resulted in a significantly accelerated wound closure in our study. Further experiments revealed that the stimulation of the in vitro wound healing by the TLR2 agonist was dependent on cathepsins. Interestingly, inhibition of TLR2 has been shown to reduce the CTSS gene expression in human endothelial cells, supporting our data [36]. Our observation is of major importance, because it links the experiments of this study with our previous findings [20]. Moreover, since we used a TLR2 agonist and an unspecific cathepsin inhibitor, this result may have an even broader significance for the understanding of periodontal diseases, because periodontitis is not only caused by a single bacterium or mediated by a single member of the cathepsin family. Whether the increased wound closure in the presence of the TLR2 agonist, as observed in our experiments, might be an attempt of the cells to maintain tissue homeostasis, has to be clarified in further studies.”

We have added the following information to the Conclusions section (page 11; lines 1-2):

“In addition, cathepsins might be exploited by periodontal bacteria to regulate critical PDL cell functions.”

We have added the following references to the Reference list (reference numbers 3, 25, 26, 36; pages 13, 15, 16; lines 5-7, 10-14, 12-14):


We have added the following information to the Figure legends (page 18; lines 21-26):

“Fig. 4

(A) Average wound closure of PDL cell monolayers in the presence or absence of a TLR2 agonist (Pam3CSK4; 1 μg/ml) over 24 h. Mean ± SEM. * significant (p<0.05) difference between groups. (B) Average wound closure of PDL cell monolayers treated with a TLR2 agonist in the presence and absence of a cathepsin inhibitor (Z-FA-FMK; 50 μM) over 24 h. Mean ± SEM, * significant (p<0.05) difference between groups.”

7. Concern of the reviewer:

Maybe the authors want to consider some of the suggestions but they should at least strengthen their introduction and discussion removing all the textbook knowledge that is not directly relevant for the study. Too little is written about the molecular mechanisms how CatS can exert its function in physiology and pathology.

Our response:

We understand the reviewer’s concern regarding the too comprehensive presentation of basic knowledge in the Introduction and Discussion sections. Wherever possible, we have removed text passages in order to make the manuscript more concise. Furthermore, we have added more details about molecular mechanisms of CTSS.

We have added the following information to the Discussion section (pages 8-10; lines 18-20, 18-19, 28-1, 4):

“CTSS has been broadly implicated in health and pathology including autoimmune diseases, allergic inflammation and asthma, diabetes and obesity, cardiovascular and pulmonary diseases as well as cancer [33].”
“Interestingly, inhibition of TLR2 has been shown to reduce the CTSS gene expression in human endothelial cells, supporting our data [36].”

“Interestingly, CTSS has the ability to degrade CTSK, indicating complex interactions between both cathepsins [40].”

“…TLR2-mediated p38/Akt signaling activation and…”

We have added the following references to the Reference list (reference numbers 33, 36, 40; page 16; lines 3-4, 12-14, 22-23):


8. Concern of the reviewer:

Would be nice to do a LPS- or ligature-induced periodontitis model in CatS KO mice.

Our response:

We completely agree with the reviewer and are grateful for this comment.

We have added the following sentence to the Discussion section (page 10; lines 20-22):

“Moreover, in order to further explore the role of CTSS in a more complex environment, an experimental periodontitis model in CTSS knock-out mice might be helpful.”

Again, we are very grateful to the reviewer for his valuable time as well as the very constructive comments.