The effect of elastase and its inhibition by sivelestat in equine endometrosis

Subjects: Zoology
Contributor: Ana Amaral

Metallopeptidases (MMP-2 and -9) are enzymes involved in ECM remodeling. The modulation of elastase-induced deleterious effect on ECM and MMPs could be important for the prevention of fibrosis development. The selective inhibitor sivelestat is known to inhibit elastase activity.

Keywords: endometrosis ; mare ; elastase ; sivelestat ; collagen ; metallopeptidases ; endometrium ; neutrophil extracellular traps (NETs)

1. Introduction

After breeding, mares develop a transient physiological endometritis, which resolves shortly in healthy uteri. The semen-induced uterine inflammation is characterized by a fast arrival of neutrophils into the uterine lumen. [1][2] The influx of inflammatory cells in the mare's uterus empowers the inflammatory reaction, resulting in the removal of unnecessary spermatozoa, contaminating bacteria, and debris. [3][4] In addition, active neutrophils at the inflammation site also release their DNA and cytoplasm proteins to the extracellular environment, such as histones, and proteases as elastase (ELA), cathepsin G (CAT), and myeloperoxidase (MPO), forming neutrophil extracellular traps (NETs).[5][6] Equine neutrophils produce NETs in the mare endometrium in the presence of *Escherichia coli* and *Streptococcus equi* subspecies *zooepidemicus* [7], or in contact with equine semen. [8][9] However, the proteases found in NETs might also induce a pro-fibrotic response in the endometrium of mares susceptible to chronic endometritis (endometrosis), characterized by the accumulation of collagen type I (COL1), which may link these proteases to endometrosis pathogenesis.[10][11]

After tissue injury, for extracellular matrix (ECM) reorganization, and especially in the presence of continuous stimuli, the parenchymal tissue is replaced by connective tissue components, such as interstitial COL1.[12] If the balance between ECM synthesis and degradation fails, it leads to fibrosis and to an increase in ECM components' deposition and/or a reduction of its degradation. Metallopeptidases (MMPs) are proteases involved in ECM balance maintenance. Among them, MMP-2 and MMP-9 are enzymes that denature collagens (gelatins) and other ECM substrates.[13] However, it has been documented that MMPs can have both stimulatory or inhibitory effects in fibrosis and can act differently among organs.[14] Our previous studies showed that the endometrial expression of MMPs and their tissue inhibitors (TIMPs) is altered at the different stages of endometrosis, and in response to interleukins.[15][16]

Elastase is a serine protease that has been reported to be increased in neutrophils retrieved from the sputum of cystic fibrosis patients[17], and to induce in vitro lung fibroblast proliferation and myofibroblast differentiation. [18] Recently, we have found that ELA induced *COL1A2* mRNA transcripts [10][11] and COL1 relative abundance [10] in equine endometrium explants, suggesting ELA´s involvement in the development of equine endometrosis.

The use of sivelestat sodium salt (SIV), which is a selective inhibitor of ELA retrieved from neutrophils, has shown beneficial effects on fibrosis impairment, either during in vitro studies or in clinical trials. Sivelestat has been reported to reduce pulmonary deposition of COL and fibrosis in mice [19], and to diminish the in vitro *COL1A2* transcription in equine endometrium .[11] In addition, SIV administration in human patients with acute lung injury has improved their clinical condition and prognosis[20][21]. Altogether, the inhibition of the pro-fibrotic effects of ELA by SIV in several fibrotic diseases in a number species substantiate the use of SIV as a potential therapeutic approach for equine endometrosis. Thus, the aim of this *in vitro* study was to evaluate the inhibitory effect of SIV on ELA induced COL1 protein relative abundance in equine endometrial explants, and the effect of ELA and SIV on the expression and activity of MMP-2 and MMP-9.

2. Results

The present study showed that ELA is capable of inducing *COL1A2* mRNA transcription by mare endometrial tissue *in vitro*, in both FP and MLP. Moreover, the inhibitory effect of SIV on ELA-induced *COL1A2* transcripts was observed in FP and MLP equine endometrium. Thus, SIV might be a helpful inhibitor of ELA induced COL1 production in equine endometrium by reducing *COL1A2* gene transcription, and its use in fighting fibrosis may be considered as a putative therapeutic approach.

Endometria with mild/moderate endometrosis lesions (category IIA/IIB) showed different *MMP2* and *MMP9* mRNA levels and protein activity in response to ELA or SIV treatments, either alone or combined, depending on the treatment length. These findings suggest that hormonal changes and duration of the stimulus can affect the endometrial response.

3. Conclusion

The present data support the hypothesis that the protease ELA present in NETs is capable of inducing *COL1* mRNA transcription in equine endometrium and might be an important player in the regulatory cascade of the pathogenesis of endometrosis in mares. This fibrogenic action is inhibited by ELA selective inhibitor SIV, which may provoke a reduction in COL1 production by the mare endometrium. Moreover, further studies are needed to understand the cellular mechanisms and pathways leading to endometrosis, and the process in which MMP-2 and MMP-9 are involved. The complexity of equine endometrosis suggests that effective therapeutic interventions may require the administration of more than one agent, capable of inhibiting fibrosis in a nonspecific way. The promising results of the present work might be the basis for future development of putative therapeutic means to impair endometrosis.

References

1. T. Kotilainen; M. Huhtinen; T. Katila; Sperm-induced leukocytosis in the equine uterus. *Theriogenology* **1994**, *41*, 629-636, 10.1016/0093-691x(94)90173-g.
2. Terttu Katila; Onset and Duration of Uterine Inflammatory Response of Mares after Insemination with Fresh Semen. *Biology of Reproduction* **1995**, *52*, 515-517, 10.1093/biolreprod/52.monograph_series1.515.
3. M. H. Troedsson; Function of uterine and blood-derived polymorphonuclear neutrophils in mares susceptible and resistant to chronic uterine infection: phagocytosis and chemotaxis. *Biology of Reproduction* **1993**, *49*, 507-514, 10.1095/biolreprod49.3.507.
4. Mats H.T. Troedsson; Breeding-Induced Endometritis in Mares. *Veterinary Clinics of North America: Equine Practice* **2006**, *22*, 705-712, 10.1016/j.cveq.2006.07.003.
5. V. Brinkmann; Neutrophil Extracellular Traps Kill Bacteria. *Science* **2004**, *303*, 1532-1535, 10.1126/science.1092385.
6. Selina K Jorch; Paul Kubes; An emerging role for neutrophil extracellular traps in noninfectious disease. *Nature Medicine* **2017**, *23*, 279-287, 10.1038/nm.4294.
7. M.R. Rebordão; C. Carneiro; Graça Alexandre-Pires; P. Brito; C. Pereira; T. Nunes; António Galvão; Alexandre B. Leitão; C. Vilela; Graça Ferreira-Dias; et al. Neutrophil extracellular traps formation by bacteria causing endometritis in the mare. *Journal of Reproductive Immunology* **2014**, *106*, 41-49, 10.1016/j.jri.2014.08.003.
8. Abdorrahman S. Alghamdi; Douglas N. Foster; Seminal DNase Frees Spermatozoa Entangled in Neutrophil Extracellular Traps. *Biology of Reproduction* **2005**, *73*, 1174-1181, 10.1095/biolreprod.105.045666.
9. Abdorrahman S. Alghamdi; Bethany J. Lovaas; Scott L. Bird; G. Cliff Lamb; Aaron K. Rendahl; Patrick C. Taube; Uglas N. Foster; Species-specific interaction of seminal plasma on sperm–neutrophil binding. *Animal Reproduction Science* **2009**, *114*, 331-344, 10.1016/j.anireprosci.2008.10.015.
10. Maria Rosa Rebordão; Ana Amaral; Karolina Łukasik; Anna Szóstek-Mioduchowska; Pedro Pinto-Bravo; António Galvão; Dariusz J. Skarzynski; Graça Ferreira-Dias; Constituents of neutrophil extracellular traps induce in vitro collagen formation in mare endometrium. *Theriogenology* **2018**, *113*, 8-18, 10.1016/j.theriogenology.2018.02.001.
11. Ana Amaral; Carina Fernandes; Karolina Łukasik; Anna Szóstek-Mioduchowska; Agnieszka Baclawska; Maria Rosa Rebordão; Joana Aguiar-Silva; Pedro Pinto-Bravo; Dariusz J. Skarzynski; Graça Ferreira-Dias; et al. Elastase inhibition affects collagen transcription and prostaglandin secretion in mare endometrium during the estrous cycle. *Reproduction in Domestic Animals* **2018**, *53*, 66-69, 10.1111/rda.13258.

12. Thomas A Wynn; Common and unique mechanisms regulate fibrosis in various fibroproliferative diseases. *Journal of Clinical Investigation* **2007**, *117*, 524-529, 10.1172/JCI31487.
13. Jennifer Vandooren; Philippe E. Van Den Steen; Ghislain Opdenakker; Biochemistry and molecular biology of gelatinase B or matrix metalloproteinase-9 (MMP-9): The next decade. *Critical Reviews in Biochemistry and Molecular Biology* **2013**, *48*, 222-272, 10.3109/10409238.2013.770819.
14. Matthew Giannandrea; William C. Parks; Diverse functions of matrix metalloproteinases during fibrosis. *Disease Models & Mechanisms* **2014**, *7*, 193-203, 10.1242/dmm.012062.
15. Anna Szóstek-Mioduchowska; A. Baclawska; K. Okuda; Dariusz J. Skarzynski; Effect of proinflammatory cytokines on endometrial collagen and metallopeptidase expression during the course of equine endometrosis. *Cytokine* **2019**, *123*, 154767, 10.1016/j.cyto.2019.154767.
16. Anna Szóstek-Mioduchowska; Mariola Słowińska; Joanna Pacewicz; Dariusz J. Skarzynski; Kiyoshi Okuda; Matrix metallopeptidase expression and modulation by transforming growth factor-β1 in equine endometrosis. *Scientific Reports* **2020**, *10*, 1-14, 10.1038/s41598-020-58109-0.
17. A. Susanne Dittrich; Iris Kühbandner; Stefanie Gehrig; Verena Rickert-Zacharias; Matthew Twigg; Sabine Wege; Clifford C. Taggart; Felix Herth; Carsten Schultz; Marcus A. Mall; et al. Elastase activity on sputum neutrophils correlates with severity of lung disease in cystic fibrosis. *European Respiratory Journal* **2018**, *51*, 1701910, 10.1183/13993003.01910-2017.
18. Alyssa D. Gregory; Corrine R. Kliment; Heather E. Metz; Kyoung-Hee Kim; Julia Kargl; Brittani A. Agostini; Lauren T. Crum; Elizabeth A. Oczypok; Tim A. Oury; A. McGarry Houghton; et al. Neutrophil elastase promotes myofibroblast differentiation in lung fibrosis.. *Journal of Leukocyte Biology* **2015**, *98*, 143-52, 10.1189/jlb.3HI1014-493R.
19. Akihiro Takemasa; Yoshiki Ishii; Takeshi Fukuda; A neutrophil elastase inhibitor prevents bleomycin-induced pulmonary fibrosis in mice. *European Respiratory Journal* **2012**, *40*, 1475-1482, 10.1183/09031936.00127011.
20. Naoki Aikawa; Akitoshi Ishizaka; Hiroyuki Hirasawa; Shuji Shimazaki; Yasuhiro Yamamoto; Hisashi Sugimoto; Masahiro Shinozaki; Nobuyuki Taenaka; Shigeatsu Endo; Toshiaki Ikeda; et al. Reevaluation of the efficacy and safety of the neutrophil elastase inhibitor, Sivelestat, for the treatment of acute lung injury associated with systemic inflammatory response syndrome; a phase IV study. *Pulmonary Pharmacology & Therapeutics* **2011**, *24*, 549-554, 10.1016/j.pupt.2011.03.001.
21. Takashi Kido; Keiji Muramatsu; Kazuhiro Yatera; Takeshi Asakawa; Hiroki Otsubo; Tatsuhiko Kubo; Yoshihisa Fujino; Shinya Matsuda; Toshihiko Mayumi; Hiroshi Mukae; et al. Efficacy of early sivelestat administration on acute lung injury and acute respiratory distress syndrome. *Respirology* **2016**, *22*, 708-713, 10.1111/resp.12969.
