Reduced Levels and Bioactivity of Endogenous Protease Cathepsin D in Genital Tract Secretions of Postmenopausal Women

Mariel Jais,¹ Naji Younes,¹ Stacey Chapman,² Susan Cu-Uvin,^{2,3} and Mimi Ghosh¹

SEXUALLY ACTIVE OLDER ADULTS are an emerging high-risk group for HIV acquisition and transmission,¹ yet few studies have focused on genital tract immunity in postmenopausal women as it pertains to HIV susceptibility. Endogenous proteases and antiproteases (P/A-P) are integral components of genital secretions and function during routine tissue remodeling in wound healing, menstrual cycling, and pregnancy.² Recent data indicate that P/A-P axis might be associated with inflammation and, therefore, an important determinant of HIV susceptibility.³ In this study, we investigated proteases cathepsins B, D, and G, and neutrophil elastase (NE) in genital secretions of postmenopausal women.

Twenty women who had regular menses for the past 3 months comprised the premenopausal group and 20 women who did not have menses for at least 1 year comprised the postmenopausal group. The age range for premenopausal women was 23–48 years (mean 35). For postmenopausal women, age range was 45–81 years, (mean 55). Each premenopausal woman provided three samples coinciding with proliferative (days 5–9), ovulatory (days 12–16), and secretory (days 19–23) stages of their menstrual cycle (with day 0 signifying start of menses). Each postmenopausal woman provided one sample. Both groups had similar percentage of bacterial vaginosis (BV) (nine visits for premenopausal versus eight visits for postmenopausal). However, three visits of *Trichomonas vaginalis* (TV) were all in premenopausal group with two of those with both BV and TV. Cohort characteristics and sample collection procedures are described in Ref. ¹

Cervical vaginal lavage (CVL) was assayed for cathepsins B, D, and G by ELISA (R&D Systems, Minneapolis, MN; ABCAM, Cambridge, MA; Abnova, Taipei City, Taiwan, respectively). NE concentrations were determined using a multiplex bead immunoassay system (MILLIPLEX Human Sepsis Magnetic Bead Panel 3; EMD Millipore, Billerica, MA). Protease bioactivity for cathepsins B and D was measured using fluorescence-based assays (ABCAM).

Values less than lower limit of detection were assigned the value of “1” and all values were log transformed. As premenopausal women have repeated mediator measures, all comparisons were based on generalized estimating equations models.

We observed significantly lower levels of cathepsin D in postmenopausal women than in premenopausal women (median, premenopausal 4.2 vs. postmenopausal 0 log pg/ml, $p \leq 0.0001$) (Fig. 1A). Although no significant changes across menstrual cycle were observed, there was a trend toward higher levels during the proliferative phase, which has also been observed by other studies.⁴ Cathepsin D bioactivity was also significantly reduced in postmenopausal women (median, premenopausal 3 fluorescence units (FU) vs. postmenopausal 2 FU, $p = .004$) (Fig. 1B) without any significant changes across menstrual cycle. In contrast, although we detected high levels and bioactivity of cathepsin B, no significant differences were observed by menopausal or menstrual status (not shown). Cathepsin G was undetectable in all samples. High levels of NE were detected and a trend toward lower values during the ovulatory stage was observed, as previously described by Tawara *et al.*⁵ However, no significant changes were observed by menstrual or menopausal status (not shown). Furthermore, there were no correlations between levels or activities of proteases and BV or TV status (not shown).

Cathepsin D has been shown to be associated with enhanced HIV infection/replication in vaginal secretions.⁶ However, our study found significantly reduced levels and activity of cathepsin D in postmenopausal CVL. This matches with our recently published data from the same cohort showing reduced levels of other genital immune mediators in postmenopausal women.¹ Further studies are needed to understand mechanisms of HIV acquisition/transmission in postmenopausal women.

Acknowledgments

Funding for this study was provided by R03 A1102837-01(M.G.), GWU Start-up funds (M.G.). We would like to thank Dr. Phalguni Gupta and Deena Ratner at the University of Pittsburgh for providing HIV-1 virus stocks; Kim Rapozoa and Jaclyn Kurpewski at Miriam Hospital Research Center, Brown University, for participant recruitment and sample processing; Dr. Charles Wira and his group at Geisel School of Medicine at Dartmouth for advice on data analysis and article writing. We thank the District of Columbia

¹Department of Epidemiology and Biostatistics, The George Washington University, Washington, District of Columbia.

Departments of ²Medicine and ³Obstetrics and Gynecology, Alpert School of Medicine, Brown University, Providence, Rhode Island.

FIG. 1. Reduced levels and bioactivity of cathepsin D in CVL from postmenopausal women. CVL from premenopausal ($n=60$) and postmenopausal ($n=19$) women was tested for cathepsin D by ELISA. **(A)** Cathepsin D levels in premenopausal women (proliferative, ovulatory, and secretory stages of menstrual cycle) compared with those in postmenopausal women. Data are presented as pg/ml of protein. Bars depict median, interquartile range, and 95% CIs. **(B)** Cathepsin D bioactivity was measured in CVL using a fluorescence-labeled synthetic substrate and compared between premenopausal (proliferative, ovulatory, and secretory stages of menstrual cycle) and postmenopausal women. Bars depict median, interquartile range, and 95% CIs. CI, confidence interval; CVL, cervical vaginal lavage; FU, fluorescence units.

(AI117970) and the Lifespan/Tufts/Brown (P30AI042853) Center for AIDS research for mentorship and support.

Author Disclosure Statement

No competing financial interests exist.

References

- Jais M, Younes N, Chapman S, Cu-Uvin S, Ghosh M: Reduced levels of genital tract immune biomarkers in postmenopausal women: Implications for HIV acquisition. *Am J Obstet Gynecol* 2016;215:324.e1–324.e10.
- Salamonsen LA: Tissue injury and repair in the female human reproductive tract. *Reproduction* 2003;125:301–311.
- Birse K, Arnold KB, Novak RM, McCorrister S, Shaw S, Westmacott GR, *et al.*: Molecular signatures of immune activation and epithelial barrier remodeling are enhanced during the luteal phase of the menstrual cycle: Implications for HIV susceptibility. *J Virol* 2015;89:8793–8805.

4. Laudanski P, Gorodkiewicz E, Ramotowska B, Charkiewicz R, Kuzmicki M, Szamatowicz J: Determination of cathepsins B, D and G concentration in eutopic proliferative endometrium of women with endometriosis by the surface plasmon resonance imaging (SPRI) technique. *Eur J Obstet Gynecol Reprod Biol* 2013;169:80–83.
5. Fumiko T, Naoaki T, Nobuhiko S, Naohiro K: Changes in cervical neutrophil elastase levels during the menstrual cycle. *Reprod Med Biol* 2012;11:65.
6. El Messaoudi K, Thiry L, Van Tieghem N, Liesnard C, Englert Y, Moguevsky N *et al.*: HIV-1 infectivity and host

range modification by cathepsin D present in human vaginal secretions. *AIDS* 1999;13:333–339.

Address correspondence to:

Mimi Ghosh

Department of Epidemiology and Biostatistics

The George Washington University

950 New Hampshire Avenue

Washington DC 20052

E-mail: mghosh@gwu.edu