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Doxycycline alters the expression of matrix metalloproteases in the endometrial cells exposed to ovarian steroids and pro-inflammatory cytokine

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Abstract

Evidence suggests that doxycycline (Dox), acting through an anti-inflammatory mechanism, inhibits expression of matrix metalloproteinases (MMPs). Since the endometrial environment in contraceptive users experiencing breakthrough bleeding is characterized by elevated production of MMPs, we examined the effect of Dox on endometrial expression of MMPs using an in vitro model consisting of endometrial glandular epithelial cells (GEC), stromal (ESC) cells, and an endometrial surface epithelial cell line (HES). GEC, ESC and HES maintained under defined culture conditions expressed variable levels of MMP-2 and MMP-9, and Dox in a dose-dependent manner (1–50 μg/ml) reduced the production of proMMP-2 after 24 h treatment ($P < 0.05$). Dox (25 μg/ml), alone or in combination with 17β estradiol (E2), medroxyprogesterone acetate (MPA) and E2 + MPA (10^{-8} M), as well as TNF-α (25 ng/ml) in cell- and time-dependent manners, moderately altered the expression of MMP-2 and MMP-9 mRNA in GEC, ESC and HES compared to untreated controls ($P < 0.05$). Dox, either alone or in combination with ovarian steroids and TNF-α, reduced production of pro-MMP-2 and proMMP9, as well as TIMP-1 and TIMP-2, without affecting the level of active MMPs produced by these cells ($P < 0.05$). In conclusion, the results indicate that Dox only moderately and in a cell-specific manner reduces expression of MMPs without influencing their activity, suggesting that Dox's therapeutic benefits in controlling irregular breakthrough bleeding in contraceptive users occurs site specifically and possibly through a mechanism involving MMPs and TIMPs expression.

Keywords: Uterine bleeding; Endometrium; Doxycycline; Proteases; Cytokine

1. Introduction

Irregular breakthrough bleeding commonly observed in women using different contraceptive systems, specifically progestin-only contraceptives, is associated with loss of endometrial tissue integrity with apparent matrix degradation and vascular fragility (for review, see Jabbour et al., 2006). It is widely accepted that matrix metalloproteinases (MMPs) act as key regulators of extracellular matrix (ECM) turnover. Through this activity, MMPs influence the outcome of inflammatory reaction, angiogenesis and tissue remodeling, and cause the release of ECM-bound growth factors and cytokines that regulate many of these processes (Mott and Werb, 2004). The expression of MMPs is highly regulated at several levels, including transcription, translation and proteolytic activities. However, loss of regulation of expression and activity has been directly linked to a number of degenerative disorders such as rheumatoid

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arthritis, osteoarthritis and chronic wound healing (Mott and Werb, 2004). The proteolytic activity of MMPs is controlled, at least in part, by tissue inhibitors of MMPs (TIMPs) which consist of TIMP-1 to TIMP-4 and are expressed in a wide range of cells and tissues (Jiang et al., 2002).

The expression of several MMPs and TIMPs has been documented in human endometrium displaying a highly regulated profile throughout the menstrual cycle (Curry and Osteen, 2003; Zhang and Nothnick, 2005). Such highly regulated expression and proteolytic activity of MMPs is altered in women experiencing irregular uterine breakthrough bleeding due to contraceptive use, specifically progestin-only contraceptive systems compared to normal menstrual cycle (Marbaix et al., 2000; Vincent et al., 2002; Rhoton-Vlasak et al., 2005; Jabbour et al., 2006). Although the molecular mechanism to explain such an alteration is unclear, ovarian steroids and several proinflammatory cytokines, such as interleukins, tumor necrosis factor alpha (TNF-α) and angiogenic factors, have been shown to regulate the expression of MMPs in endometrial cells (Marbaix et al., 2000; Vincent et al., 2002; Chegini et al., 2003; Curry and Osteen, 2003; Jabbour et al., 2006; Malik et al., 2006). For instance, progesterone suppresses, while IL-1 and TNF-α often increase, the production and proteolytic activity of MMPs in endometrial stromal and epithelial cells (Marbaix et al., 2000; Jabbour et al., 2006).

Currently, there is no effective treatment to control uterine breakthrough bleeding in contraceptive users, and options included ethinyl estradiol, ibuprofen, oral contraceptives and levonorgestrel (for review, see Jabbour et al., 2006). In recent years, doxycycline (Dox) and several other tetracycline analogues have been used therapeutically in disorders that are characterized by high levels of protease activity, including osteoarthritis, rheumatoid arthritis and adult periodontitis. The beneficial effects of Dox appear to occur through non-antimicrobial mechanisms, including inhibition of MMP's proteolytic activities (Ramamurthy et al., 2002; Siemonsma et al., 2003; Lee et al., 2004a,b; Smith and Cook, 2004; Onoda et al., 2004; Salvi and Lang, 2005). Previous studies have also identified Dox as an effective therapy in women with pelvic inflammatory disorders and premenstrual syndrome (Toth et al., 1988; Walters and Gibbs, 1990; Heinonen and Leinonen, 2003). A recent pilot study has reported that Dox therapy reduced the number of bleeding/spotting days in women experiencing breakthrough bleeding because of a progesterone-only contraceptive (Weisberg et al., 2006). Furthermore, in a mouse model of endometrial breakdown due to progestin-treatment, Dox reduces the activity of MMP-2, -8 and -9 without affecting endometrial tissue integrity (Kaitu'u et al., 2005). Using endometrial cells as an *in vitro* model we have shown that Dox altered the expression of several proinflammatory cytokines and chemokines, including those that regulate expression of MMPs (Li et al., 2006).

In this study, we have used an *in vitro* model consisting of isolated human endometrial glandular epithelial and stromal cells and human endometrial surface epithelial cell line (HES) maintained under defined culture conditions to determine the endometrial action of Dox on MMPs and TIMPs expression. Under such conditions, the cells were exposed to Dox, or in combination with medroxyprogesterone acetate (MPA), 17β estradiol (E_2) and E_2 + MPA, as well as TNF-α, representing an endometrial environment exposed to contraceptives and inflammatory conditions, respectively.

2. Material and methods

Endometrial glandular epithelial (GEC) and stromal (ESC) cells were isolated from small portions of endometrial tissues obtained from premenopausal women, ranging in age from 22 to 41 years, who were undergoing hysterectomy for medically indicated reasons (excluding endometrial cancer and leiomyoma) at the University of Florida-affiliated Shands Hospital. These patients were not under any hormonal treatments at the time of surgery. Tissues were collected after obtaining approval from the University of Florida Institutional Review Board, without requiring written informed consent. GEC and ESC were isolated and cultured in DMEM-Ham's F-12 supplemented with 10% FBS, as previously described in detail (Chegini et al., 1992). The purity of cell preparations was determined in freshly isolated cells and after the first passage by immunostaining for cytokeratin (epithelial), vimentin (stromal) and α smooth muscle actin (smooth muscle), using their respective antibodies (Chegini et al., 1992). Cells were then cultured in 75 mm flasks in the presence of 10% FBS until reaching visible confluence and used in experiments between the first and fourth passage. The human endometrial surface epithelial cell line (HES) was kindly provided by Dr. D. Kniss (Ohio State University, Columbus, OH) and cultured as previously described (Luo et al., 2004).

All materials for realtime PCR and ELISA were purchased from Applied Biosystem (Foster City, CA) and R&D System (Minneapolis, MN). ELISA kits measuring active MMP-2 and MMP-9 were purchased from (GE Healthcare Bio-Sciences Corp. formerly Amersham, Piscataway, NJ). 17β estradiol (E2), medroxypro-