Biomarkers, May 2008; 13(3): 257–269

informa
healthcare

ICAM-1 expression in vaginal cells as a potential biomarker for inflammatory response

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Abstract

This study aimed to elucidate the mechanisms that may lead to an efficient strategy to induce a suitable host response of the vaginal mucosa upon exposure to intravaginally delivered exogenous compounds. It was hypothesized that the upregulation of intercellular adhesion molecule (ICAM)-1 gene expression may reflect the inflammatory response evoked by exogenous compounds. Major emphasis was placed on ethylenediamine tetraacetic acid (EDTA) which was added as a synergistic agent to conventional spermicidal agents or anti-HIV drugs. The levels of ICAM-1 mRNA were examined as a surrogate marker for inflammatory response in human vaginal epithelial cells upon exposure to EDTA or interleukin (IL)-1β (i.e. positive control, 25 mM). The effects of estrogen on EDTA-induced ICAM-1 expression were also evaluated for the estrogen involvement in the inflammatory process of the vaginal mucosa. ICAM-1 expression in human vaginal cells (VK2/E6E7 cells) increased as EDTA concentration added to human vaginal cell lines increased. The effects of estrogen on EDTA-induced ICAM-1 expression in human vaginal epithelial cells were estrogen-concentration dependent; estrogen at lower concentrations (~1–10 nM) did not affect ICAM-1 expression, whereas estrogen at higher concentrations (~100 nM–1 μM) attenuated ICAM-1 expression. The influence of estrogen in ICAM-1 expression suggests the beneficial effects of estrogen on the regulation of vaginal homeostasis. Identification and quantification of specific surrogate markers for the inflammatory response evoked by exogenous compounds and their regulation by estrogen will lead to an efficient strategy against sexually transmitted diseases including AIDS.

Keywords: *Exogenous compounds, ICAM-1, estrogen, VK2/E6E7 cells, sexually transmitted diseases*

(Received 10 July 2007; accepted 4 December 2007)

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ISSN 1354-750X print/ISSN 1366-5804 online
DOI: 10.1080/13547500701843338

Introduction

A successful application of a barrier device to patients depends on an accurate assessment of pharmacological efficacy and biocompatibility. Since inflammation of the female reproductive tract increases susceptibility to viral pathogens, such as human immunodeficiency virus (HIV), human papilloma virus (HPV) and other viral infections (Fichorova et al. 2001a, 2001b, 2004), organ-specific responses to extracellular stimuli should be thoroughly monitored. An excessive release of proinflammatory cytokines may alter the mucosal balance between tissue integrity and the regulation process, leading to enhanced penetration and replication of viral pathogens (Wagner & Levine 1978, Fichorova et al. 2004, Bowie & Haga 2005). Because cell toxicity may be associated with transient expression of markers of immune activation by dying cells, cytotoxicity caused by the intravaginal barrier system can be assessed by quantifying surrogate markers for immune response.

Intercellular adhesion molecule (ICAM)-1 is a member of the immunoglobulin supergene family of adhesion proteins that serve as the counter-receptor for leukocyte function-associated antigen-1. ICAM-1 functions as a stimulatory molecule for effector cells (Satoh et al. 1994). It was found that protein kinase C-δ (PKC-δ) and the p38 mitogen-activated protein (MAP) kinase pathway play an essential role in thrombin-induced ICAM-1 gene expression of endothelial cells (Rahman et al. 2001). It was also reported that the nuclear factor-κB signalling cascade, which has been involved in Ad5-dependent nuclear transcription, affected the long-term activation of ICAM-1 (De Cesaris et al. 1999, Roebuck & Finnegan 1999, Vielma et al. 2003). ICAM-1 expression has been demonstrated in the immortalized human vaginal epithelial cell line model (Fichorova et al. 1999, 2001a). ICAM-1 expression in human bone or fibroblasts seemed to be pathological status-dependent and correlated with interleukin (IL)-6 and prostaglandin (PG) E_2 production (Maldonado et al. 2003, Beddy et al. 2004, Lavigne et al. 2004). These previous results have led to the hypothesis that the upregulation of ICAM-1 gene expression may reflect inflammatory response evoked by exogenous compounds including microbicides.

Since ethylenediamine tetraacetic acid (EDTA) was added as a synergistic agent to conventional spermicides or anti-HIV drugs, its effects on mucosal biocompatibility have been examined previously (Lee et al. 1996, Warrier et al. 2004). The Western immunoblot and immunohistochemistry studies on the rabbit vaginal mucosa following exposure to EDTA revealed differential expression and topological localization of Ca-regulated proteins, such as MAP kinases (ERK, JNK and p38) along with inducible-type nitric oxide synthetase (iNOS) and p53 (Choe et al. 2004). The results of this study indicated that EDTA activated MAP kinases in the vaginal mucosa through the modulation of the calcium gradient. It was known that the transcriptional factor MAP kinase signalling cascade affected the long-term activation of ICAM-1. Subsequently, in the present study, ICAM-1 was used as a surrogate marker for inflammatory response of the vaginal mucosa evoked by EDTA (~0–3.5%).

Estrogen has been effective in attenuating various symptoms including ischaemic heart disease, osteoporosis, reduction in cognitive function and macular degeneration, and provides a significant improvement in quality of women's lives. Before menopause, women are relatively protected against various diseases including vaginal dryness and cardiovascular complications through hormone regulation (Schneider 2002). Serum levels of ICAM-1 were inversely correlated with estrogen levels in

women and studies have shown that postmenopausal women treated with hormone replacement therapy (HRT) or estrogen alone had reduced ICAM-1 and vascular cell adhesion molecule (VCAM)-1 serum levels compared with untreated postmenopausal women (Colacurci et al. 2003). Moreover, it was reported that some women remained HIV uninfected despite having repeated intercourses with HIV-positive partners (Brewer et al. 2003), which has led to the investigation on factors associated with abnormalities in HIV progression and transmission among the discordant couples. Therefore, the effects of estrogen on the expression of ICAM-1 in the vaginal cells were evaluated to present evidence for abnormalities involved with penetration and replication of viral pathogens. The linkages between estrogen at various concentrations and inflammatory response caused by exogenous compounds were further established.

The aim of this study was to elucidate the mechanism that may lead to an efficient strategy to induce a suitable host response of the vaginal mucosa against exogenous compounds. EDTA was tested as a candidate exogenous compound at its higher concentrations (~3.5%) which may occur due to the burst release from intravaginal formulations or accumulation at local sites. We evaluated the levels of ICAM-1 mRNA as a surrogate marker for inflammatory response of human vaginal epithelial cells upon exposure to EDTA (~0–3.5%) or IL-1β (i.e. positive control, 25 mM). Cyclooxygenase (COX)-2, whose immune response is estrogen-concentration dependent (Egan et al. 2005), was added as a comparative marker for exogenous compound-induced immune response. It was subsequently determined whether or not estrogen effects on ICAM-1 expression were estrogen-concentration dependent (~1 nM–1 μM).

Materials and methods

The model cell lines

It was previously demonstrated that cell lines of human immune and epithelial origin can be used as appropriate surrogates for primary cells in investigating the effects of exogenous compounds on cell metabolism, membrane composition and integrity, and the effects of cell type, proliferation and differentiation on microbicide sensitivity (Krebs et al. 2002, Guseva et al. 2005). Normal vaginal epithelial cell line, VK2/E6E7 (CRL-11882 (ATCC)), was used in this study. The major characteristics of VK2/E6E7 and HeLa cell lines (both from ATCC, Manassas, VA, USA) and their comparison with the vaginal tissues are described in Table I. HeLa cell line, which is a well-established and easily available human cervical adenocarcinoma cell line, is also included in Table I for comparison purposes.

Identification of the estrogen receptor in vaginal cell lines

Because estrogen receptors (ER) regulate genes involved in tissue growth and differentiation (Scobie et al. 2002), any correlations between expressions of ERα and ICAM-1 levels may delineate the role of estrogen in the inflammatory response of the vaginal mucosa. ER in the vaginal cell lines were identified to demonstrate that the selected model cell line was suitable for delineating the effects of estrogen on the inflammatory response of the vaginal mucosa. ERα were identified from the protein expression profiles of VK2/E6E7 cell lines using antibody PA1-309 (Santa Cruz Biotechnology, Santa Cruz, CA, USA) through Western blot analysis. Antibody

Table I. Characteristics of cervicovaginal cell lines.

Name	HeLa	VK2/E6E7	Vaginal tissue
Type	Human cervical adenocarcinoma	Human vagina (epithelial)	
Growth media	Minimum essential medium FBS	Keratinocyte-SFM EGF, BPE and $CaCl_2$	
Tight junctions	–	+	+
Involucrin	–	+	+
Estrogen receptor	β	α and β	α and β
Cytokins 19	+	+	+
Cytokins 10,13,16	–	+	+
Cytokins 8,18	+	+	–
Secretory protein	+	+	+

Data from Fichorova et al. 1997, 1999, 2001a, 2001b, Bodnar et al. 1983, Bootman 1996, Elkeeb et al. 2003, Pivarcsi et al. 2005.

immunizing peptide corresponds to amino acid residues 21–32 from human ERα (Hu et al. 2005).

Effects of estrogen on cell viability of the vaginal cell lines

Cell viability has been an integral part of the assessment process of materials designed to be implanted into host tissues (Kirkpatrick et al. 1998) or intended for antiestrogenic agents (Varma & Conrad 2002). The cell viability was evaluated based on the principle of E-screen technique, which compares the number of cells in the ER-positive cell line following a long-term incubation period in the presence or absence of estrogenic substances in medium (Zachareswki 1997). Cell Titer 96 AQueous assay kit (Promega, Madison, WI, USA) was used to measure the viability of VK2/E6E7 by quantifying formazan, a coloured product, which is converted from MTT by NADPH or NADH in metabolically active cells (Pauwels et al. 1988, Berridge & Tan 1993).

Briefly, VK2/E6E7 were harvested at 70% confluence and about 2×10^6 cells per well were seeded in a 96-well plate loaded with keratinized serum-free medium (K-SFM; Gibco, Carlsbad, CA, USA) containing bovine pituitary extract (BPE) (50 μg ml^{-1}), human recombinant epidermal growth factor (EGF) (0.1 ng ml^{-1}), $CaCl_2$ (0.4 mM), penicillin (100 U ml^{-1}) and streptomycin (100 μg ml^{-1}). They were incubated at 37°C under 5% CO_2 and 95% humidity. After 24 h, the medium in the wells was replaced with the testing medium containing EDTA (~0.035–3.5%) or 17β-estradiol (~1 nM–1 μM, Sigma, St Louis, MO, USA), which were chosen based on the criteria previously reported (Wiese et al. 1992, Nagira et al. 2006). Estrogen vehicles at the concentrations of 1, 10, and 100 and 1000 nM were prepared from a stock solution (10 mM estradiol in 100% ethanol). The maximal concentration of the estrogen vehicle (1000 nM) contains the maximal ethanol concentration of 0.01% (upon 1:10000 dilution from a stock solution of 100% ethanol) which did not affect ICAM-1/COX-2 expression or cell viability.

EDTA was prepared with the cell culture medium and 17β-estradiol was diluted with the cell culture medium from a stock solution (dissolved in 100% ethanol). The drug-free cell culture medium was used as the control. In the experiments with 17β-estradiol and EDTA, the plate was incubated at 37°C for 24 h and then the medium in the wells was replaced with 100 μl of the medium containing 20 μl of the Cell Titer

96 AQueous assay solution. The wells loaded with the drug-free medium were used as the background. The plate was incubated at 37°C/5% CO_2 for 4 h and the absorbance of the formazan products was read using a spectrofluorimeter reader at 485 nm.

Identification and quantification of ICAM-1 in the vaginal cell lines

The changes in expression of specific proteins or enzyme activities have been used to elucidate the effects of estrogenic substances on the concentration-dependent immune response. ICAM-1 was selected as a biomarker for the inflammatory response of the vaginal mucosa evoked by exogenous compounds including EDTA. As previously described, VK2/E6E7 cells were cultured in a 24-well tissue culture plate at a density of 2×10^6 cells per well. When cells reached the confluence, the initial medium was replaced with K-SFM containing EDTA (0, 0.035, 0.1 and 0.35%) or IL-1β (25 mM used as a positive control). After 6 h treatment, endothelial ICAM-1 expression was analyzed using reverse transcriptase–polymerase chain reaction (RT-PCR) and Western blot analyses. The period of 6 h was selected based on the optimal wearing time of a barrier device. Genes for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were used as the internal control. Primers for the ICAM-1 gene were 5′-CCT GTT TCC TGC CTC TGA AG-3′ (sense) and 5′-GTC TGC TGA GAC CCC TCT TG-3′ (antisense), and those for the GAPDH gene were 5′-CCA TGG AGA AGG CTG GG-3′ (sense) and 5′-CAA AGT TGT CAT GGA TGA CC-3′ (antisense).

Effects of estrogen on ICAM-1 expression in the cell line mode

The expression levels of ICAM-1 in the vagina epithelial cells upon exposure to various concentrations of estradiol have been examined to elucidate dose-dependent effects of estrogenic compounds on the cell response. COX-2 was also examined for comparison purposes, as its immune response was reported to be estrogen-concentration dependent.

VK2/E6E7 cells were cultured in a 24-well tissue culture plate at a density of 2×10^6 cells per well as previously described. Cells were seeded on the 100 mm Petri dishes in the density of 1 million per dish. Estradiol solutions (~1 nM–1 μM) and IL-1β (25 mM, the positive control) were prepared in K-SFM. When cells reached the confluence, the initial medium was replaced with K-SFM containing estradiol in the mixture with EDTA (0.1%) or IL-1β (25 mM). After 6 h treatment, RT-PCR and Western blot studies were performed on the samples to examine the changes in the expression level of ICAM-1.

Western blot/RT-PCR studies

For the Western blot study, proteins were separated by SDS-PAGE and transferred to a nitrocellulose membrane. ICAM-1 antibody, COX-2 antibody, β-actin antibody and HRP-conjugated antirabbit IgG (Cell Signaling Technology, Beverly, MA, USA) were used for the Western blot analysis. The blots were drained and exposed to Pierce blue film. Each staining was quantified based on the optical density of each peak, which was calculated as a ratio against the highest peak (Densitometric Analysis).

For the RT-PCR study, total RNA was prepared from cells using Trizol reagent. Single-strand cDNA were made from 1 μg total RNA by reverse transcription using a random primer. PCR was performed with ICAM-1 and COX-2 primer through

35 cycles of 94°C for 30 s, 55°C for 1 min, and 72°C for 1 min. The PCR products were separated using electrophoresis on agarose gel in the presence of ethidium bromide. The RT-PCR study was carried out according to the standard protocol using the Reverse Transcription Kit (Protégé Inc, Madison, WI, USA) and DNA Thermal cycle (Midwest Scientific, St Louis, MO, USA). GAPDH was used as a reference. The gel was exposed to UV and pictures were taken using the digital camera.

Statistical analysis

The differences in protein expression and changes in cell viability under various experimental conditions were statistically analyzed by a one-way analysis of variance (ANOVA) with pairwise multiple comparisons using the Student–Newman–Keuls method. *P*-values ≤ 0.05 were considered to be statistically significant.

Results

Identification of the estrogen receptor in the vaginal cell lines

Of special interest among EDTA-extracted proteins is the one with the molecular weight of 66 kDa, which was identified as ERα through the Western blot analysis. The results of Western blot analysis revealed that there were differences in the intensity of protein bands between the control and EDTA-treated samples (Figure 1). The expression of ERα steadily increased as the exposed estrogen concentration to VK2/E6E7 cells increased. The results of this study confirmed the presence of ER (66 kDa) in the vaginal cell line model (i.e. VK2/E6E7 cells), warranting further studies on the linkage between the ER-mediated inflammatory regulation and EDTA-induced ICAM-1 expression.

Cell viability assays

The cell viability assay was performed to determine whether or not EDTA or estrogen affects the cell proliferation rate. As shown in Figure 2,17β-estradiol maintains cell

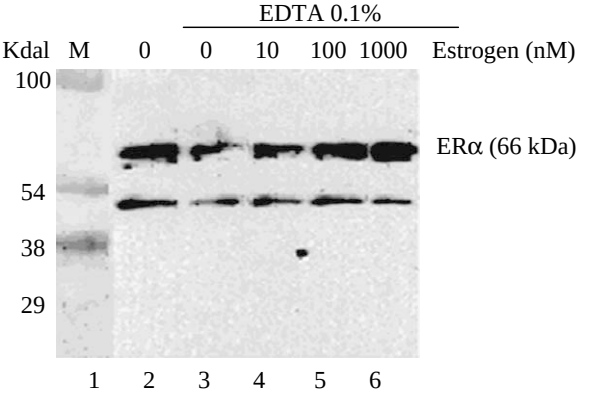

Figure 1. Expression of estrogen receptor α (66 kDa) in vaginal cell lines (VK2/E6E7). Lane 1, molecular weight marker; lane 2, control; lane 3, 0.1% EDTA; lane 4, 0.1% EDTA+10 nM estradiol; lane 5, 0.1% EDTA+100 nM estradiol; lane 6, 0.1% EDTA+1000 nM estradiol. The second lane is β-actin (45 KDa).

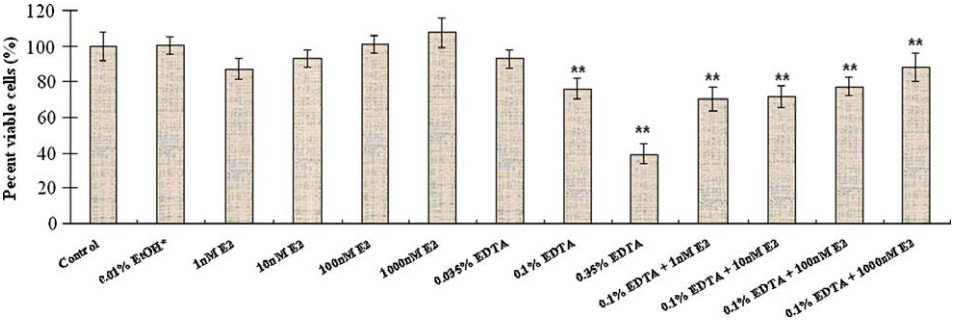

Figure 2. Effects of estrogen and EDTA on vaginal cell viability. Cells are exposed to estrogen or EDTA for 6 h ($n=6$; the number of experiments). The bars represent means and error bars represent SD. *Concentration of ethanol in the sample of 1000 nM estradiol. **$p<0.05$ versus control.

viability at all tested concentrations (~1 nM–1 μM). Ethanol concentration in the estrogen vehicles (the maximal concentration of 0.01% on 1:10 000 dilution from a stock solution of 100% ethanol) did not affect cell viability.

EDTA at a concentration of 0.035% did not affect the VK2/E6E7 cell viability, whereas EDTA at concentrations of 0.1% and 0.35% reduced the percentage amount of viable cells by about 30% and 60%, respectively. The results of these studies indicate that exogenous compounds such as EDTA affect the cell viability, and thus, a proper selection of the loading dose in the system is essential for the safe and efficient application of intravaginal drug carriers.

The percentage amount of viable cells was further determined upon exposure to EDTA (0.1%) in the presence of 17β-estradiol. There were no attenuation or deterioration effects of estrogen at concentrations of 1–10 nM on the cell viability evoked by EDTA (Figure 2). However, estradiol at higher concentrations (100 nM and 1000 nM) significantly protected the cell viability. The results of this study confirmed that estrogen at higher concentrations (>100 nM) has beneficial effects on cell viability.

Effects of EDTA on ICAM-1 expression

The level of ICAM-1 expression was used as a biomarker for immune response of the vaginal mucosa potentially evoked by exogenous compounds loaded in the intravaginal formulation. As shown in Figures 3 and 4, RT-PCR and Western blot studies revealed that mRNA expression of ICAM-1 in VK2/E6E7 cells were stimulated by EDTA in a concentration-dependent manner, whereas COX-2 (74 kDa) was not significantly overexpressed by EDTA until its concentration reached at 0.35%. As shown in Figure 4, while the 92 kDa protein of ICAM-1 (upper band) was not expressed differentially, the 89 kDa protein of ICAM-1 (lower band) was gradually induced by EDTA treatment. The 89-kDa fragment of ICAM-1 was not distinctively recognized by the antibody probably due to the lack of specificity for this fragment. The optical density of each peak obtained by densitometric analysis (Choe et al. 2004) also confirmed that immune reactivity of ICAM-1 gradually increased as the EDTA concentration increased (Figure 4). As dose-dependent effects of estrogen appeared more prominent on the expression of ICAM-1 than that of COX-2, ICAM-1 seems to

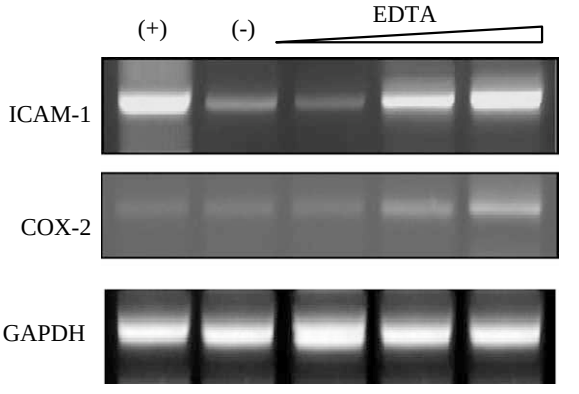

Figure 3. RT-PCR results of ICAM-1 and COX-2. Each lane was reverse transcribed from 2 μg of total RNA. EDTA treatments were 0%, 0.035%, 0.1%, 0.35%, and (+) indicates positive control treated with 25 mM IL-1β.

be a suitable marker in vaginal epithelial cells for the evaluation of the inflammatory response evoked by exogenous compounds loaded in the intravaginal barrier devices.

Effects of estrogen on ICAM-1 expression

The effects of externally added estrogen on ICAM-1 expression in the vaginal epithelial cells were evaluated to demonstrate the involvement of estrogen in the inflammatory process. Estrogen concentrations ranging from 1 nM to 1 μM were tested to determine whether or not there were concentration-dependent effects of estrogen on ICAM-1 expression.

As shown in Figures 5 and 6, ICAM-1 expressions in VK2/E6E7 cells upon exposure to the mixture solution of EDTA (0.1%) and estradiol (1 nM) or estradiol (10 nM) remained constant, whereas ICAM-1 expressions upon exposure to the same concentration of EDTA (0.1%) in the presence of estradiol (100 nM or 1000 nM) were significantly reduced. These results confirmed that ICAM-1 expressions in the vaginal epithelial cells upon exposure to higher concentrations of estrogen were

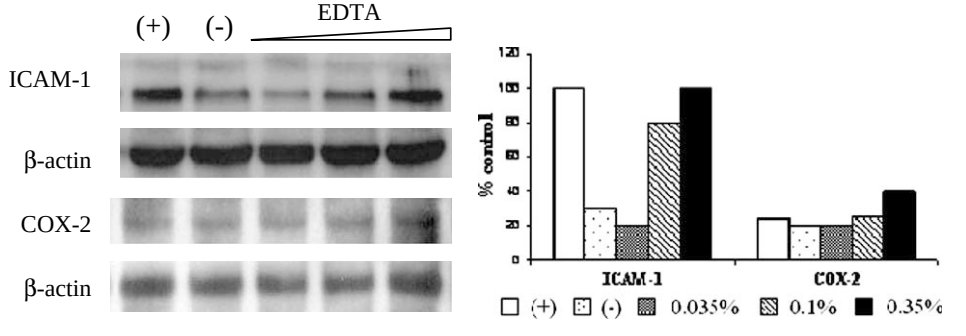

Figure 4. Western blot analysis of ICAM-1 and COX-2 expressions. Each lane was loaded with 10 μg of total protein. Left, while the 92 kDa protein of ICAM-1 (upper band) was not expressed differentially, the 89 kDa protein of ICAM-1 (lower band) was gradually induced by EDTA treatment. EDTA treatments were 0%, 0.035%, 0.1%, 0.35% and (+) indicates positive control treated with 25 mM IL-1β. Right, optical density of ICAM expressions quantified by densitometer ($n = 3$; the number of experiments).

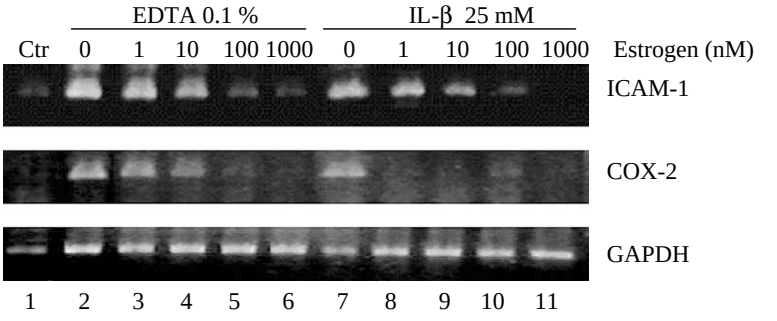

Figure 5. RT-PCR results of ICAM-1 and COX-2 upon exposure to EDTA (0.1%) or estrogen (1–1000 nM) for 6 h. The expression of ICAM decreased as the exposed estrogen concentration increased.

lowered or attenuated. This finding is in good accord with the previous cell viability tests, in which estrogen at the same concentration either did not affect (1 nM and 10 nM) or protected (1000 nM) cell viability. This finding is also paralleled with the previous results on the profiles of ERα expression in which estrogen maintained the level of ERα at a normal range as compared with the lowered level caused by EDTA. The results of this study clearly demonstrated that estrogen-attenuated inflammatory response caused by EDTA in the vaginal epithelial cells and estrogen's effects on ICAM-1 expression were estrogen-concentration dependent.

Discussion

This study focused on the host response of the vaginal mucosa against intravaginally delivered exogenous compounds and its regulation by estrogen. Recent progress in biotechnology and proteomics made it possible to concomitantly examine the status of pharmacological activities and host response upon exposure to the exogenous compounds.

Upregulation of ICAM-1 gene expression evoked by exogenous compounds was shown in the very initial phase of infection (Ledebur & Park 1995). ICAM-1 expression has been demonstrated at mostly endothelial cells. It was reported that the vaginal cell line VK2/E6E7 responds to infection with *Neisseria* by upregulation of distinct proinflammatory cytokines as well as ICAM-1 (Fichorova et al. 2001a). In cultured

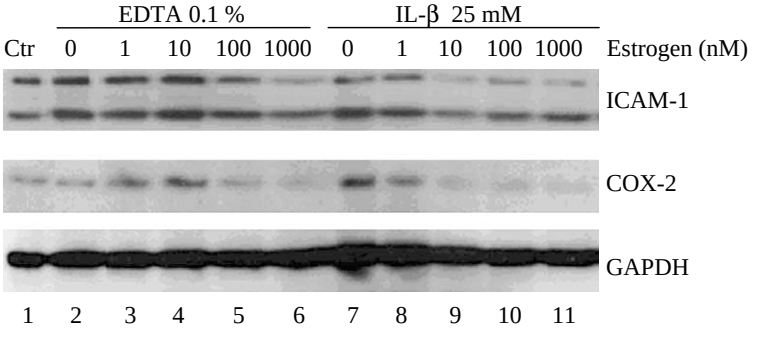

Figure 6. Western blot analysis of ICAM-1 and COX-2 upon exposure to EDTA (0.1%) or estrogen (1–1000 nM) for 6 h.

human umbilical vein endothelial cells (HUVECs), tumour necrosis factor-α and lipopolysaccharide increased phosphorylation of p38 MAPK (P-p38 MAPK) and ICAM-1 expression (Ju et al. 2003). ICAM-1 was expressed on apical membrane of both colonocytes and HT29 cells in response to supraphysiological cytokine concentrations (Vainer et al. 2003). Significant increases in both protein and mRNA levels of ICAM-1 gene expression in primary cultured human nasal epithelial cells (HNECs) after stimulation by glucocorticosteroids were also observed (Shirasaki et al. 2004). ICAM-1, vascular adhesion protein-1 (VAP-1) and P-selectin were expressed on the vaginal mucosa (Johansson et al. 1999). Soluble ICAM-1 was detected in the cervicovaginal fluid of women in preterm labour, in which elevated sICAM-1 concentrations predicted short intervals to delivery with high specificity (Marvin et al. 2000).

The previous reports along with the finding that ICAM-1 was expressed on endothelium cells and recently also on epithelial cells (Cheeti et al. 2006) warranted further investigation on the effects of exogenous compounds on the early signalling events in relation to the upregulation of the inflammatory adhesion molecule (i.e. ICAM-1). The results of the present study support the hypothesis that the upregulation of ICAM-1 in the vaginal epithelial cells is affected by intravaginally exposable exogenous compounds. Subsequent studies in animal models and human volunteers will be needed to confirm these results under *in vivo* conditions.

The effects of sex hormones on the host response of the vaginal mucosa were further evaluated by examining ICAM-1 expression in the human vaginal cells upon exposure to the mixture of EDTA and estrogen. The presence of ERα in the model cell lines and its regulation by estrogen warranted investigation on immune response of the vaginal cells against exogenous compounds. ER is a large super family of ligand-dependent intracellular proteins that stimulate transcription of specific genes by binding to specific DNA sequences following activation by the appropriate hormone (Metivier et al. 2004). The ER complex can mediate the activation of oncogenes, proto-oncogenes, nuclear proteins and other target genes that can be involved in the transformation of normal to cancerous cells. ER has been classified into two distinct isoforms, α and β. ERα is known to interact with several known co-activators in a ligand-dependent and -independent manner to enhance transcriptional activity of target genes (Scobie et al. 2002). The functional interplay between different domains of ERα is responsible for the overall properties of the full-length protein. The corresponding genes for the ERα and β are *NR3A1* and *NR3A2*, respectively (Ji et al. 2005).

Estrogen profoundly affects the onset and severity of various immune-mediated inflammatory processes (Bowden et al. 2006). A variety of studies showed that the effects of estrogen on ICAM-1 or COX-2 were exposure-site specific and estrogen-concentration dependent. VCAM-1 levels were significantly higher in transdermal HRT users than in fertile women (Farzati et al. 2002). On the other hand, studies have shown that postmenopausal women treated with the chronic oral route of HRT or estrogen alone had reduced ICAM-1 and VCAM-1 serum levels as compared with untreated postmenopausal women and that the serum levels of ICAM-1 were inversely correlated with the estrogen levels (Colacurci et al. 2003). 17β-Estradiol enhanced insulin-induced tyrosine phosphorylation of insulin receptor substrate-1 (IRS-1), IRS-1/p85 association and 2-deoxyglucose uptake at 10^{-8} M, but lowered these activities at 10^{-5} M, indicating that estradiol affects the metabolic action of insulin in a concentration-dependent manner (Nagira et al. 2006).

COX-2 is added as a comparative marker, which may surrogately reflect EDTA- or exogenous compound-induced immune response. COX-2, expressed in vascular endothelial cells, catalyzes the formation of prostaglandin endoperoxide from arachidonic acid, which subsequently is transformed to PGI_2 (prostacyclin), a major arachidonic acid-derived COX product in endothelial cells. Selective COX-2 inhibitors impeded the excretion of PGI_2 metabolites (Korita et al. 2004) and reduced oxidant stress and platelet activation that contribute to atherogenesis in female mice (Egan et al. 2005). These mechanisms seem to be relevant to estrogen-involved protection in women since estrogen increases the expression of COX-2 in vascular tissues and promotes *in vitro* production of PGI_2 (Shah 2005). Judging from the results in which the expressions of ICAM-1 were more prominently EDTA-concentration specific than those of COX-2, ICAM-1 appeared to be a better surrogate marker for the inflammatory response of vaginal cells.

In drug-induced autoimmunity, estrogen-dependent regulation in the expression of the surrogate marker was directly associated with increased inflammatory severity (Gillgrass et al. 2005). The present study also revealed that estrogen attenuated vaginal inflammatory responses and abrogated EDTA-mediated effects on the expression of ICAM-1. The involved mechanisms in which inflammatory suppression may be mediated through proinflammatory mediators need further investigation. The results of these studies should be of great importance with respect to the potential beneficial effects of estrogen on the inflammatory response of the vaginal epithelial cells caused by exogenous compounds. Subsequently, the optimal concentration of estrogen needed to protect women against exogenous compound-induced inflammation should be identified to establish the most efficient strategy against sexually transmitted disease, including AIDS.

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