SEXUAL MEDICINE

BASIC SCIENCE

All-Trans Retinoic Acid Increases Aquaporin 3 Expression in Human Vaginal Epithelial Cells

Hyun-Suk Lee, MS,[1,*] Sun-Ouck Kim, MD,[1,*] Kyuyoun Ahn, MD, PhD,[2] and Kwangsung Park, MD, PhD[1]

ABSTRACT

Introduction: Water channel aquaporin 3 (AQP3) is an aquaglyceroporin that transports small neutral solutes and water. All-trans retinoic acid (ATRA), a member of the retinoid drug class, acts as a regulator in several biological processes.

Aim: To investigate the effect of ATRA on the expression of AQP3 in human vaginal epithelial cells.

Methods: Human vaginal mucosal epithelial cells (CRL2616) were treated with ATRA 0, 0.01, 0.1, and 1 μmol/L for 24 hours to examine the dose-dependent effects of ATRA and with ATRA 1 μmol/L for 0, 3, 6, 12, and 24 hours.

Main Outcome Measures: The expression of AQP3 and retinoic acid receptor (RAR) was determined by western blot analysis and reverse transcription polymerase chain reaction.

Results: AQP3 was detected in the cell membrane of human vaginal epithelial cells. ATRA increased the protein expression and mRNA levels of AQP3 in a dose-dependent manner ($P < .05$). ATRA also increased the protein expression of RARα ($P < .05$). Treatment of CRL2616 cells with an RAR antagonist (Ro 41-5253) significantly decreased AQP3 protein expression ($P < .05$).

Conclusion: ATRA mediated by RARα increased AQP3 gene and protein expression in human vaginal mucosal epithelial cells. These results imply that AQP3 regulated by ATRA could play an important role in the mechanism of vaginal lubrication.

Key Words: All-Trans Retinoic Acid; AQP3; Human Vaginal Cell

INTRODUCTION

Female sexual dysfunction is a common problem in women. Laumann et al[1] reported that 43% of women experience sexual problems that negatively affect their quality of life. The physiologic mechanisms of genital sexual arousal are not clearly understood. The female sexual arousal response has been suggested to be a complex physiologic process featuring clitoral and vaginal engorgement and vaginal lubrication as a result of increased clitoral and vaginal blood flow.[2–4] Vaginal lubrication is one indicator of the genital sexual arousal response. However, the underlying mechanisms of vaginal lubrication are not clear. The vaginal mucosa includes a complex network of small capillaries just beneath the vaginal epithelium that could account for vaginal fluid secretion and lubrication.[5]

Aquaporins (AQPs) are traditionally known as membrane proteins that facilitate water movement across cells.[6] In some cases, AQPs can transport small solutes such as glycerol, ions, and gas. Among the AQP family members, AQP3 transports glycerol and presumably other small solutes across cells.[7,8] Vaginal fluid consists of various elements besides water, and we previously reported the distinct localization of AQPs in the vagina of rats and humans as a potential component of vaginal lubrication.[9,10] Thus, we targeted AQP3 as a potential active mediator of vaginal lubrication.

All-trans retinoic acid (ATRA) is an important mediator of several biological processes in the body, such as differentiation, proliferation, embryogenesis, reproduction, and apoptosis, and

Received February 29, 2016. Accepted July 4, 2016.

[1]Department of Urology, Chonnam National University Medical School, Sexual Medicine Research Center, Chonnam National University, Gwangju, Korea;

[2]Department of Anatomy, Chonnam National University Medical School, Sexual Medicine Research Center, Chonnam National University, Gwangju, Korea;

*Equivalent contribution.

http://dx.doi.org/10.1016/j.esxm.2016.07.001

immune-mediated anti-inflammatory effects.[11–13] Retinoic acid (RA) is reported to be an active regulator of body fluid secretion, and RA might be involved in maintaining the homeostasis of bronchial surface fluid secretion.[14–16] We hypothesized that ATRA and AQP3 might be involved in vaginal fluid regulation. In support of this hypothesis, ATRA was reported to increase the expression of AQP3 and enhance biological activity in human skin.[17] In that study, topical application of ATRA induced a meaningful response of epidermal AQP3 and strong immunoreactivity in the epidermal basal layers, suggesting possible involvement of ATRA in the regulation of AQP3.

AIMS

In the present study, we investigated whether ATRA regulates AQP3 in human vaginal epithelial cells to assess the functional implications of AQP3 in vaginal lubrication.

METHODS

Cell Culture and Treatment

Human vaginal mucosal epithelial (CRL-2616) cells were obtained from the American Type Culture Collection (Rockville, MD, USA). Cells were incubated at 37°C in 5% CO_2 and maintained in keratinocyte serum-free medium (Gibco BRL, Paisley, UK). Cells were used when they reached a confluent state.

ATRA was obtained from Sigma (St Louis, MO, USA). For the in vitro studies, stock solutions of ATRA were prepared in dimethyl sulfoxide and subsequent dilutions were prepared in culture medium, so that the final concentration of dimethyl sulfoxide was always 0.1%. ATRA at concentrations of 0, 0.01, 0.1, and 1 μmol/L was applied to CRL2616 cells for 24 hours. The RA receptor-α (RARα) antagonist, Ro41-5253, was purchased from Sigma. Cells were allowed to attach for 24 hours and then were incubated in medium with the appropriate concentration of Ro 41-5253 (0.1–5 μmol/L).

Immunocytochemistry

Cells were rinsed in phosphate buffered saline and then treated with normal chicken serum for 30 minutes to block non-specific binding. After washing in phosphate buffered saline, cells were incubated with antibodies for AQP3 (1:100; Abcam, Cambridge, MA, USA) in phosphate buffered saline for 12 to 14 hours at 4°C. Immunoreactivity for AQP3 was detected using Alexa Fluor 594-conjugated chicken anti-rabbit immunoglobulin G (H + L; Molecular Probes, Inc, Eugene, OR, USA). Cells were mounted in a mounting solution containing 4′-6-diamidino-2-phenylindole. Cells were examined with an LSM 510 (Carl Zeiss, Seoul, Korea) confocal microscope with an excitation wavelength appropriate for the Alexa Fluor dyes (405 and 594 nm). Final images were constructed using LSM Image Examiner software.

Sodium Dodecylsulfate Polyacrylamide Gel Electrophoresis and Western Blot

Proteins were electrophoresed on sodium dodecylsulfate 10% polyacrylamide gels and transferred to a polyvinylidene difluoride membrane (Amersham Pharmacia Biotech, Little Chalfont, UK). The blots were washed with Tris-buffered saline and Tween-20 (Tris-HCl 10 mmol/L, pH 7.6, NaCl 150 mmol/L, 0.05% Tween-20). The membrane was blocked with 5% skimmed milk for 1 hour and incubated with the appropriate primary antibody at the dilution recommended by the supplier. Rabbit α-AQP3 (1:1,000; Calbiochem, Nottingham, UK), rabbit α-RARα (1:1,000; Santa Cruz Biotechnology, Santa Cruz, CA, USA), and glyceraldehyde 3-phosphate dehydrogenase (1:10,000; Cell Signaling, Danvers, MA, USA) were used. The membrane was washed, and the primary antibodies were detected with goat anti-rabbit immunoglobulin G conjugated to horseradish peroxidase. Antibody incubations were performed at 4°C in an incubator. Bands were visualized using enhanced chemiluminescence (Amersham Pharmacia Biotech).

Reverse Transcription Polymerase Chain Reaction

Total RNA from CRL2616 pellets was extracted by the acid guanidium-thiocyanate-phenol-chloroform method using Tri-Reagent. The concentration of total RNA was determined by measuring the optical density at 260 nm. One microgram of total RNA was converted into first-strand cDNA using the ImProm-II Reverse Transcription system with Oligo(dT)15 primers, as suggested by the manufacturer's instructions (Promega, Madison, WI, USA). For the semiquantitative polymerase chain reaction experiment, the reverse transcription product was amplified using specific primers designed from human AQP3 cDNAs and β-actin cDNA. Amplified polymerase chain reaction products were subjected to electrophoresis on 1.5% agarose gels and visualized on an ultraviolet table.

The AQP3 forward and reverse primers were 5′-ACCTTT GCCATGTGCTTCCT-3′ and 5′-GCGTCTGTGCCAGGGT GTA-3′, respectively, and the β-actin forward and reverse primers were 5′-CTGGCACCCAGCACAATG-3′ and 5′-GCC GATCCACACGGAGTACT-3′.

Data Analysis

Data were expressed as mean ± SD of the mean. The significance of differences among the groups was determined using analysis of variance with post hoc Tukey test. Statistical significance was set at a P value less than 0.05.

RESULTS

AQP3 Is Expressed in CRL2616 Cells

AQP3 was expressed in the cell membrane of human vaginal mucosal epithelial cells (Figure 1A).

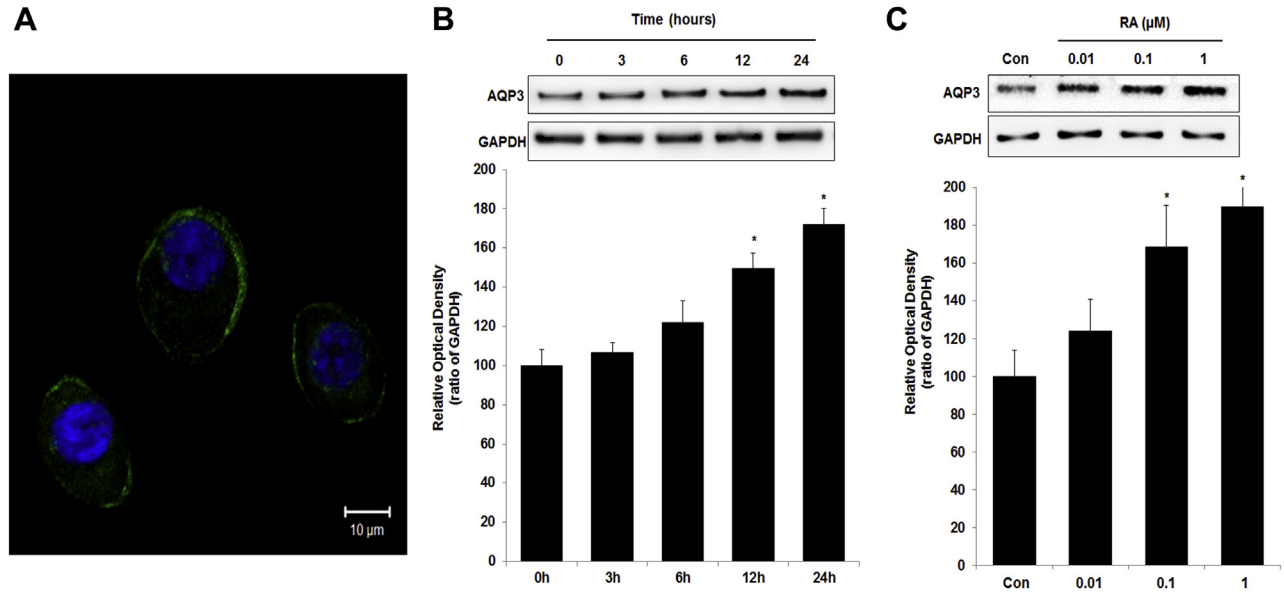

Figure 1. Effect of all-trans retinoic acid on the expression of AQP3 protein in cultured human vaginal mucosal epithelial cells. Panel A shows the detection of AQP3 in the cell membrane of human vaginal epithelial cells (green) by confocal microscopy after immunostaining with anti-AQP3. Cells also were stained with 4′-6-diamidino-2-phenylindole to visualize the nucleus (blue). Panel B shows the incubation of cells with all-trans retinoic acid 1 μmol/L for 0, 3, 6, 12, or 24 hours. Twenty micrograms of whole-cell lysates was applied to sodium dodecylsulfate polyacrylamide gel electrophoresis followed by western blotting with anti-AQP3 and anti—β-actin antibodies. Panel C shows the incubation of cells with all-trans retinoic acid 0, 0.01, 0.1, or 1 μmol/L for 24 hours. Levels of AQP3 protein were examined by western blotting. The results are representative of three independent experiments. The expression of AQP3 was dose-dependently increased by all-trans retinoic acid treatment. *$P < 0.05$ vs untreated control. AQP3 = aquaporin 3; Con = control; GAPDH = glyceraldehyde 3-phosphate dehydrogenase.

ATRA Increases AQP3 Protein Expression in CRL2616 Cells

To investigate whether ATRA affected the expression of AQP3 in human vaginal mucosal epithelial cells, CRL2616 cells were treated with ATRA 1 μmol/L for 0, 3, 6, 12, and 24 hours and AQP3 protein in whole-cell lysates was measured by western blot analysis. The expression of AQP3 increased with time, significantly so after 12 to 24 hours (Figure 1B). To examine the dose-dependent effect of ATRA, cells were treated with ATRA 0, 0.01, 0.1, and 1 μmol/L for 24 hours. ATRA increased AQP3 protein levels in a concentration-dependent manner (Figure 1C).

ATRA Upregulates AQP3 mRNA Expression in CRL2616 Cells

To analyze the effect of ATRA on the expression of AQP3 mRNA in CRL2616 cells, cells were treated with ATRA 0.01, 0.1, and 1 μmol/L for 24 hours. Similar to the AQP3 protein expression, the AQP3 mRNA expression level increased in a concentration-dependent manner after ATRA treatment (Figure 2).

ATRA Increases AQP3 Expression in CRL2616 Cells Through RAR

The effects of ATRA are mediated by its receptors, and ATRA regulates the expression of its own receptors. We examined the expression of RAR isoforms in response to ATRA treatment. Treatment of ATRA increased the expression of RARα in cells (Figure 3).

The effects of ATRA on AQP 3 expression were investigated further using an antagonist to the RAR. The antagonist (Ro 41-5253) is a synthetic retinoid that binds RARα (high affinity), RARβ, and RARγ (low affinity) and subsequently blocks binding of ATRA. Treatment of CRL2616 cells with the RAR antagonist for 24 hours significantly decreased AQP3 protein expression compared with controls, which suggested that the effects of ATRA on the expression of AQP3 were dependent on RAR (Figure 4).

DISCUSSION

In the present study, ATRA significantly increased the levels of AQP3 protein in vaginal mucosal epithelial cells. Furthermore, as shown by reverse transcription polymerase chain reaction, ATRA increased the levels of AQP3 mRNA in a dose-dependent manner. To our knowledge, this study is the first to report a possible relation between AQP3 and ATRA in the regulation of the vaginal lubrication mechanism.

The female genital arousal response is a complex physiologic process. Although the vagina does not have any gland structure, it secretes large amounts of vaginal fluid that plays an important role in vaginal lubrication.[18] Human vaginal fluid is a complex biological fluid composed of water, electrolytes,

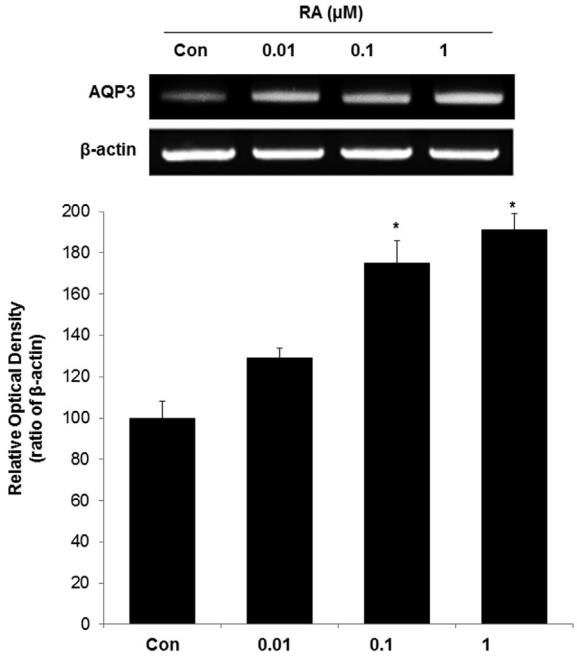

Figure 2. Effects of all-trans retinoic acid on AQP3 expression analyzed by reverse transcription polymerase chain reaction. CRL2616 cells were incubated with all-trans retinoic acid 0.01, 0.1, or 1 μmol/L for 24 hours. Then, cells were harvested and total RNA was prepared. All-trans retinoic acid dose-dependently increased AQP3 gene expression in cultured human vaginal mucosal epithelial cells. *$P < 0.05$ vs untreated control. AQP3 = aquaporin 3; Con = control; RA = retinoic acid.

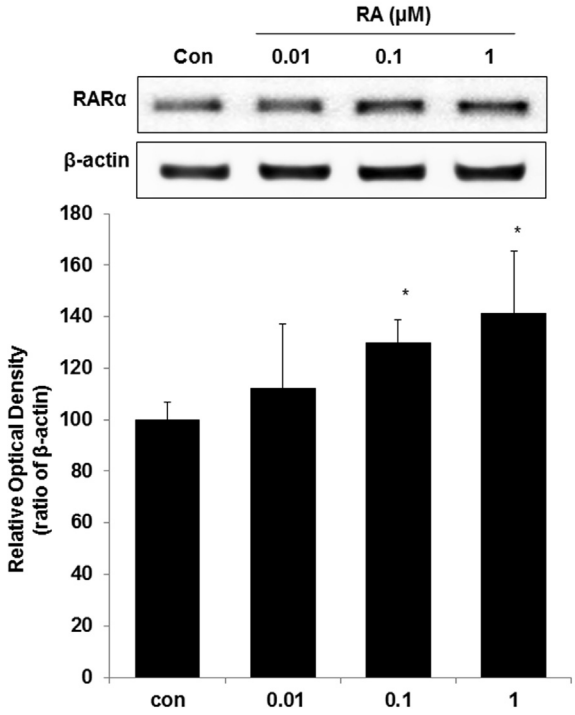

Figure 3. Expression of RARα protein after all-trans retinoic acid treatment for 24 hours in cultured human vaginal mucosal epithelial cells. Cells were incubated with all-trans retinoic acid 0.01, 0.1, or 1 μmol/L for 24 hours and levels of RARα protein were examined by western blotting with anti-RARα and anti−β-actin. There was a significant increase in the expression of RARα at the all-trans retinoic acid doses of 0.1 and 1 μmol/L. Results are representative of three independent experiments. *$P < 0.05$ vs untreated control. Con = control; RA = retinoic acid; RARα = retinoic acid receptor-α.

low-molecular-weight organic compounds (glucose, lipids, and amino acid), cells (epithelial cells, leukocytes, and lymphocytes), and some proteins and enzymes.[19] The subepithelial region of the vaginal wall includes a compact and rich microvascular network of capillaries that are an important component of vaginal lubrication.[5] The vaginal epithelium, in the basal state, reabsorbs sodium as plasma transudates from the capillaries located in the submucosal area. Levin[20] suggested that the vaginal epithelium can transport Na^+ ions from the vascular lumen to the serosal tissues, which could mediate the formation of vaginal fluid. Until now, however, the exact mechanism of vaginal lubrication has not been fully disclosed. It remains unclear whether vaginal lubrication is mediated mainly by blood flow or whether other potential mechanisms independent of blood flow also contribute.[18]

AQPs have been suggested to play an important role in vaginal lubrication.[9] In our previous animal study, we suggested a role for AQPs in vaginal lubrication by demonstrating the expression of AQP1, 2, and 3 in rat vagina.[9,21] We suggested that sexual stimulation by electric stimulation of the pelvic nerve might be involved in moving the AQP channel from the cytoplasm to the cell membrane of the epithelium and that this alteration might induce water transport across the vaginal cells. In our follow-up human study, we reported the distinct presence and immuno-localization of AQP1, 2, 3, 5, and 6 in human vaginal tissue from premenopausal women.[10] In that study, we showed that AQP water channels have different cellular localization and suggested that the AQPs might have different functional roles depending on their subtypes and cellular location.

At least 13 AQPs (AQP0−12) have been identified to date in mammalian cells, all of which are highly permeable to water. Of these, AQP3, 7, 9, and 10 also are permeable to glycerol and some solutes and are called *aquaglyceroporins*.[2] Evidence from many studies in humans and mice lacking AQPs indicates that AQPs have important physiologic roles, including facilitating trans-epithelial water transport, skin hydration, and urine concentration and regulating glandular secretion.[22,23]

In the human skin, Bellemère et al[17] investigated whether ATRA, a potent modulator of keratinocyte proliferation and differentiation, could regulate AQP3 expression in human epidermal keratinocytes. They reported that topical application of ATRA resulted in significant changes in the epidermal expression of AQP3 and powerful immunoreactivity in the epidermal layers of the skin in vivo and in vitro. They also found that the increased expression of AQP3 gene and protein induced

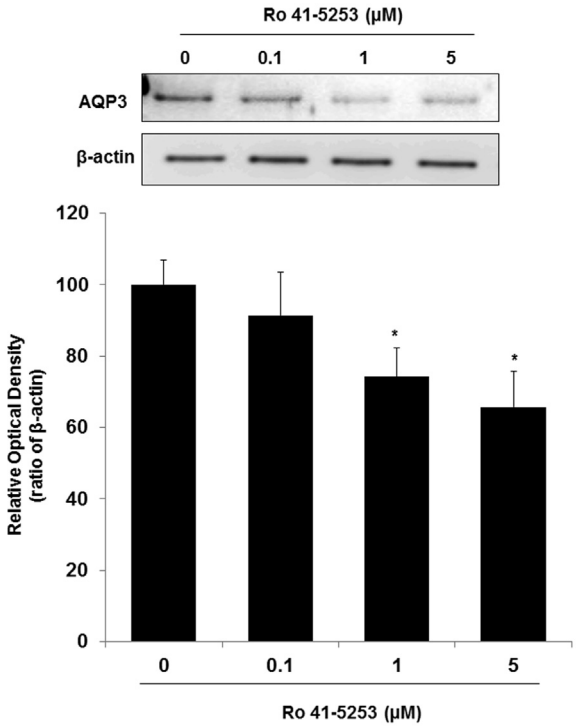

Figure 4. Expression of AQP3 after treatment with a retinoic acid receptor antagonist (Ro 41-5253). CRL2616 cells were treated with all-trans retinoic acid 1 μmol/L and different amounts of Ro 41-5253 for 24 hours. Whole-cell lysates were applied to sodium dodecylsulfate polyacrylamide gel electrophoresis followed by western blotting with anti-AQP3 and anti–β-actin antibodies. Treatment of human vaginal mucosal cells with Ro 41-5253 for 24 hours significantly decreased AQP3 protein expression compared with controls. Results represent at least three independent experiments. *$P < 0.05$ vs untreated control. AQP3 = aquaporin 3.

by ATRA was associated with increased glycerol uptake in human epidermal keratinocytes.[17]

Considering the characteristics of mucoid vaginal fluid, aquaglyceroporins are likely to be involved in the vaginal lubrication mechanism. In the present study, therefore, we focused on AQP3 because it is an aquaglyceroporin that transports glycerol and possibly other small solutes in addition to water.

ATRA is a potent stimulator of keratinocyte proliferation in skin and is usually prescribed for various skin diseases to enhance skin hydration.[24] ATRA also is involved in vaginal epithelial differentiation and proliferation. For example, ATRA deficiency in rodents results in a persistent keratinization response of the vaginal epithelium.[25] In the epithelial lining of the reproductive tracts, including the vagina, cervix, and uterus, a specific role for ATRA has been suggested in epithelial proliferation and in alteration of the pattern of keratinization.[26] The induction of gene transcription by ATRA is mediated by the ATRA receptor (RAR).[27,28] In the present study, we found that the increase in AQP3 protein stimulated by ATRA was correlated with an increase in the ATRA receptor (RARα), which suggests the active involvement of RARα in mediating the interaction between AQP3 and ATRA across the vaginal epithelial cells. To confirm the involvement of RAR, we examined the effect of RAR antagonist on the expression of AQP3 and found a significant dose-dependent decrease in AQP3 expression. These results suggest that the effect of ATRA on AQP3 expression actively involves RAR in a dose-dependent manner. In this study, we used an RAR antagonist (Ro 41-5253) that binds RARα (high affinity), RARβ, and RARγ (low affinity) and subsequently blocks binding of ATRA. Further studies are needed to evaluate the role of RAR subtypes on AQP3 expression using small interfering RNA methods.

To our knowledge, this study is the first to report a possible relation between AQP3 and ATRA in the regulation of the vaginal lubrication mechanism. Modification of AQP3 by ATRA in human vaginal epithelial cells is a new action among the numerous established effects of ATRA.

Other experimental methods for studying the actual vaginal fluid in vivo might yield further insight into the mechanism of vaginal lubrication. In our next study, we plan to study the molecular signal transduction pathway involved in the expression of AQPs with ATRA stimulation and determine whether regulating the expression of AQPs in vaginal tissue might improve vaginal lubrication in an animal model. We presume that a knockout animal model would help facilitate this investigation of the relation of these two molecules in the vaginal lubrication process. Further investigation into the effects of ATRA-induced AQP3 expression and water permeability in vivo or in vitro ultimately could provide a novel approach for elucidating the pathophysiology of vaginal secretion and for treating the sexual dysfunction of diminished vaginal lubrication. Additional studies are needed to investigate the regulation of all kinds of AQPs by ATRA with the use of vaginal cells in various clinical conditions. This goal might be accomplished by targeting specific disease conditions, such as dyspareunia, vaginal dryness, and sexual pain disorder, that might be affected by an abnormal sexual response and vaginal lubrication. Thus, the results of the present study have potential implications for future therapeutic options for the treatment of female sexual dysfunctions and abnormal vaginal lubrication.

CONCLUSIONS

The present results showed that ATRA mediated by RAR increased the expression of the AQP3 gene and protein in human vaginal mucosal epithelial cells. These results imply that AQP3 regulated by ATRA could play an important role in the mechanism of vaginal lubrication.

Corresponding Author: Kwangsung Park, MD, PhD, Department of Urology, Chonnam National University Medical School, 8 Hakdong, Donggu, Gwangju 501-757, Republic of Korea. Tel: +82-62-220-6701; Fax: +82-62-227-1643; E-mail: uropark@gmail.com

Conflict of Interest: The authors report no conflicts of interest.

Funding: This study was supported by a grant (CRI12056-21) Chonnam National University Hospital Biomedical Research Institute.

STATEMENT OF AUTHORSHIP

Category 1

(a) Conception and Design
Kwangsung Park

(b) Acquisition of Data
Hyun-Suk Lee; Sun-Ouck Kim

(c) Analysis and Interpretation of Data
Hyun-Suk Lee; Sun-Ouck Kim; Kyuyoun Ahn; Kwangsung Park

Category 2

(a) Drafting the Article
Hyun-Suk Lee; Sun-Ouck Kim; Kwangsung Park

(b) Revising It for Intellectual Content
Kyuyoun Ahn; Kwangsung Park

Category 3

(a) Final Approval of the Completed Article
Hyun-Suk Lee; Sun-Ouck Kim; Kyuyoun Ahn; Kwangsung Park

REFERENCES

1. Laumann EO, Paik A, Rosen RC. Sexual dysfunction in the United States: prevalence and predictors. **JAMA 1999;281:537-544**.
2. Goldstein I, Berman JR. Vasculogenic female sexual dysfunction: vaginal engorgement and clitoral erectile insufficiency syndromes. **Int J Impot Res 1998;10(Suppl. 2):S84-S90**.
3. Gruenwald I, Lowenstein L, Gartman I, et al. Physiological changes in female genital sensation during sexual stimulation. **J Sex Med 2007;4:390-394**.
4. Jannini EA, Buisson O, Rubio-Casillas A. Beyond the G-spot: clitourethrovaginal complex anatomy in female orgasm. **Nat Rev Urol 2014;11:531-538**.
5. Shabsigh A, Buttyan R, Burchardt T, et al. The microvascular architecture of the rat vagina revealed by image analysis of vascular corrosion casts. **Int J Impot Res 1999;11(Suppl.):S23-S30**.
6. Verkman AS. More than just water channels: unexpected cellular roles of aquaporins. **J Cell Sci 2005;118:3225-3235**.
7. Agre P, King LS, Yasui M, et al. Aquaporin water channels-from atomic structure to clinical medicine. **J Physiol 2002;542:3-16**.
8. Yasui M. Molecular mechanisms and drug development in aquaporin water channel diseases: structure and function of aquaporins. **J Pharmacol Sci 2004;96:260-263**.
9. Park K, Han HJ, Kim SW, et al. Expression of aquaporin water channels in rat vagina: potential role in vaginal lubrication. **J Sex Med 2008;5:77-82**.
10. Kim SO, Oh KJ, Lee HS, et al. Expression of aquaporin water channels in the vagina in premenopausal women. **J Sex Med 2011;8:1925-1930**.
11. Ahuja HS, Szanto A, Nagy L, et al. The retinoid X receptor and its ligands: versatile regulators of metabolic function, cell differentiation and cell death. **J Biol Regul Homeost Agents 2003;17:29-45**.
12. Wilson L, Gale E, Maden M. The role of retinoic acid in the morphogenesis of the neural tube. **J Anat 2003;203:357-368**.
13. Zhou X, Kong N, Wang J, et al. Cutting edge: all-trans retinoic acid sustains the stability and function of natural regulatory T cells in an inflammatory milieu. **J Immunol 2010;185:2675-2679**.
14. Fawzi WW, Herrera MG, Willett WC, et al. Dietary vitamin A intake and the incidence of diarrhea and respiratory infection among Sudanese children. **J Nutr 1995;125:1211-1221**.
15. Ross AC, Stephensen CB. Vitamin A and retinoids in antiviral responses. **FASEB J 1996;10:979-985**.
16. Ross DA. Vitamin A and public health: challenges for the next decade. **Proc Nutr Soc 1998;57:159-165**.
17. Bellemère G, Von Stetten O, Oddos T. Retinoic acid increases aquaporin 3 expression in normal human skin. **J Invest Dermatol 2008;128:542-548**.
18. Traish AM, Botchevar E, Kim NN. Biochemical factors modulating female genital sexual arousal physiology. **J Sex Med 2010;7:2925-2946**.
19. Dasari S, Pereira L, Reddy AP, et al. Comprehensive proteomic analysis of human cervical vaginal fluid. **J Proteome Res 2007;6:1258-1268**.
20. Levin RJ. Actions of spermicidal and virucidal agents on electrogenic ion transfer across human vaginal epithelium in vitro. **Pharmacol Toxicol 1997;81:219-225**.
21. Kim SO, Lee HS, Ahn K, et al. Effect of estrogen deprivation on the expression of aquaporins and nitric oxide synthases in rat vagina. **J Sex Med 2009;6:1579-1586**.
22. Nielsen S, Frokiaer J, Marples D, et al. Aquaporins in the kidney: from molecules to medicine. **Physiol Rev 2002;82:205-244**.
23. Verkman AS. Physiological importance of aquaporin water channels. **Ann Med 2002;34:192-200**.
24. Smit JV, de Jong EM, van Hooijdonk CA, et al. Systemic treatment of psoriatic patients with bexarotene decreases epidermal proliferation and parameters for inflammation, and improves differentiation in lesional skin. **J Am Acad Dermatol 2004;51:257-264**.
25. Van Wauwe J, Van Nyen G, Coene MC, et al. Liarozole, an inhibitor of retinoic acid metabolism, exerts retinoid-mimetic effects in vivo. **J Pharmacol Exp Ther 1992;261:773-779**.
26. Boehm N, Chateau D, Rochette-Egly C. Retinoid receptors in rat vaginal and uterine epithelia: changes with ovarian steroids. **Mol Cell Endocrinol 1997;132:101-108**.
27. Mangelsdorf DJ, Kliewer SA, Kakizuka A, et al. Retinoid receptors. **Recent Prog Horm Res 1993;48:99-121**.
28. Mangelsdorf DJ, Evans RM. The RXR heterodimers and orphan receptors. **Cell 1995;83:841-850**.