

The effects of vaginal lubricants on the human vagina: an *in vitro* analysis

Dong-Wook Park¹, Han Tae Jo², In-Ho Lee³, Chan-Woo Park³, Ju Tae Seo⁴

¹Laboratory of Reproductive Medicine, ²Department of Obstetrics and Gynecology, ³Department of Urology, Cheil General Hospital & Women's Healthcare Center, College of Medicine, Dankook University, Seoul

⁴Department of Biological Science, College of Natural Science, Ajou University, Suwon (Korea)

Summary

Purpose: Vaginal lubricants are widely used by women to resolve intercourse difficulties or as performance enhancers. However, little information is available regarding the safety of over-the-counter (OTC) lubricants. Therefore, in this study, a set of commercial lubricant products available on the Korean online market were selected and evaluated *in vitro* for their safety relevant to their osmolality. **Materials and Methods:** Five commercially available gel-based OTC lubricant products were included in this study. Osmolality was determined using a vapor pressure 5520 osmometer. Cell viability was measured by MTT assay and Western blotting. Human vaginal tissue integrity was measured by immunohistochemistry. **Results:** Four OTC lubricant showed hyper-osmolality, while one OTC lubricant was within the minimum WHO-recommended standards. The findings showed that four hyperosmolal OTC vaginal lubricants showed cellular damage to the cultured vaginal cell line and human vaginal explant. One iso-osmolal OTC vaginal lubricant showed less cellular damage to both cultured vaginal cell lines and human vaginal explants. **Conclusion:** Present study showed that the osmolality of OTC vaginal lubricant is a major factor affecting epithelial cell survival and tissue integrity. The effect of each ingredient contained in OTC lubricants was not considered. Thus, additional research is needed to evaluate formulae to enable production of safer OTC vaginal lubricants.

Key words: Vagina; Lubricant; Osmolality; Sexual problems; Tissue integrity.

Introduction

Vaginal lubricants are widely used by women to resolve intercourse difficulties or as performance enhancers [1]. Up to 46% of women between the ages of 18 to 45 years experience dyspareunia [2]. Decreased vaginal lubrication has numerous causes, including advancing age, hormonal changes, menopause, breastfeeding, stress, conditions such as inflammatory bowel disease, diabetes, chronic heart failure, and multiple sclerosis, and iatrogenic causes such as the use of radiation, chemotherapy, and antidepressants [3]. Clinicians often prescribe the use of over-the-counter (OTC) lubricants to alleviate symptoms. In the United States, it is estimated that 62% of women have used a lubricant during sexual activities and 25.3% have used a lubricant during the previous month [4].

Sexual problems are more common among infertile couples, with an incidence ranging from 5% to 55%. Sexual dysfunction can be the cause of infertility or the result of it [5, 6]. Thus, the use of vaginal lubricant can improve sexual activity and alleviate distress in infertile women. However, little information is available regarding the safety of OTC lubricants. In the United States, OTC lubricants are categorized as medical devices by the U.S. Food and Drugs Administration. In Korea, OTC lubricants are categorized

as medical devices or cosmetics. Therefore, the safety testing performed on these products is often limited. The high osmotic property of lubricants has been known as a major cause of vaginal damage and subsequent increased susceptibility for infection. The lubricants' ability to cause irritation (assessed based on the mucus production) has been reported on by Adriaens and Remon. Their report showed a significant, quadratic relationship between product osmolality and its ability to cause irritation. A hypo-osmolar lubricant caused negative mucus production, while a highly hyperosmotic lubricant resulted in severe irritation and tissue damage. An iso-osmolar lubricant caused no changes [7]. Dezzutti *et al.* showed that hyper-osmolar lubricants were associated with cellular toxicity and vaginal epithelial damage while showing no anti-viral activity [8].

In this study, a set of commercial lubricant products available on the Korean online market were selected and evaluated *in vitro* for their safety relevant to their osmolality.

Materials and Methods

Five commercially available gel-based OTC lubricant products were included in this study. All products were purchased online and the product name and manufacturer name were blinded to avoid bias on the part of the researcher regarding the products and

Revised manuscript accepted for publication September 13, 2018

to ensure fairness of evaluation.

Osmolality was determined using a vapor pressure 5520 osmometer calibrated with opti-mole 290 and 1,000 mmol/kg osmolality standards. The products were diluted ten-fold (vol/vol) with deionized water before testing since the osmometer has an upper limit of 3,200 mmol/kg.

A human vaginal epithelial cell line (VK2/E6E7 cells) was purchased from and cultured in keratinocyte-serum free medium with 0.1 ng/ml human recombinant epidermal growth factor, 0.05 mg/ml bovine pituitary extract, and 100 units/ml each of penicillin and streptomycin at 37°C with 5% CO₂ in a high humidity environment. All experiments were performed with VK2/E6E7 cells during the exponential growth phase (48 hours after plating). Lubricants were diluted with the same culture medium and applied on cultured cells with a 5% (vol/vol) final concentration over 48 hours.

Cell viability was measured using the MTT assay. Briefly, about 1×10^4 cells/well were seeded in 96-well plates and incubated in a humidified atmosphere containing 5% CO₂ overnight. After the culture medium was removed, 200 μ l of 0.5 mg/ml MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] solution was added to each well, and the mixtures were further incubated at 37°C for four hours. The reaction was stopped by replacing the MTT solution with dimethyl sulfoxide (DMSO). Formazan released from the cells was measured using a microplate reader at 570 nm.

Cells grown in plastic dishes were harvested and washed with ice-cold phosphate-buffered saline (PBS). Whole-cell lysates were obtained through lysis in the lysis buffer containing 50 mM Tris, pH 6.8, 150 mM NaCl, 5% sodium deoxycholate, 1% NP-40, and 1 mM ethylene glycol tetra-acetic acid supplemented with a mixture of protease and phosphatase inhibitors containing 100 mM 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride, 80 μ M aprotinin, 5 mM bestatin, 1.5 mM E-64, 2 mM leupeptin, and 1 mM pepstatin A. After one hour incubation on ice, the lysates were centrifuged at 13,000 rpm for 20 min at 4°C, and the protein content in the supernatant was determined using the Bio-Rad protein assay. The sample buffer containing 0.25 M Tris-HCl, pH 6.8, 4% SDS, 40% glycerol, 10% mercapto-ethanol, and 0.005% bromophenol blue was added to each whole-cell lysate, boiled for three minutes, and resolved using SDS-polyacrylamide gel electrophoresis (40 μ g protein/lane). The proteins resolved on the gel were electro-transferred onto nitrocellulose blotting membranes and immunoblotted with anti-bcl-2 (1:1000 dilution), anti-cleaved poly ADP ribose polymerase (PARP) (1:1000 dilution), anti-caspase 7 (1:1000 dilution), or anti-occludin (1:500 dilution) antibodies. The antibody-antigen complexes were visualized with appropriate HRP-conjugated secondary antibodies (1:5000 dilution) and using enhanced chemiluminescence according to the manufacturer's instructions.

Human vaginal tissue explants obtained from three independent patients who underwent routine hysterectomies were dissected and cultured as described by Introini *et al.* [9] Briefly, vaginal tissues were cut in blocks of 4-5 mm in width. Six vaginal tissues were obtained from each patient. Vaginal tissues were cultured in 37°C, 5% CO₂ incubator with RPMI 11,640 medium (5% FBS, 10% MEM- nonessential amino acids, 10% fungizone, 10% MEM-sodium pyruvate, and 1% gentamicin). Vaginal tissue blocks were cultured in the cell culture insert and the culture insert was added into the 24-well dish. The mucosal side of the vaginal tissues were placed face upwards. The medium was used to

cover the intact vaginal tissue as a control, while the mucosal sides of the other experimental tissues were covered with one lubricant each. After 48 hours of culture, tissues were fixed in 4% paraformaldehyde for 24 hours and embedded with paraffin according to the standard paraffin block method [10]. Paraffin blocks were cut in 5- μ m thickness. The paraffin sections were treated with 4% H₂O₂ for five minutes to remove the residual peroxidase. After washing with distilled water three times, the paraffin sections were incubated with 1/500-diluted primary antibody in a humidified chamber at room temperature for 12 hours. For immunohistochemistry, primary mouse monoclonal antibody against bcl-2 and cleaved caspase-7 were utilized. The sections were then reacted with secondary antibodies that are included in the LSAB-HRP detection kit for 15 minutes according to the manufacturer's instruction. After washing with PBS three times, chromogen development with diaminobenzidine was performed. The sections were then counter-stained with hematoxylin for ten seconds, sealed with Permount solution, and observed under a light microscope. All primary antibodies were diluted with a solution containing carrier proteins, such as 3% bovine serum albumin to reduce the non-specific binding. Positive staining was analyzed using Image pro plus 4.5 software. Briefly, a percentage of positive stained cells were calculated using the formula (number of positive stained cells/total number of detected cells) under the microscopic images ($\times 200$). Weak brown stains were excluded from the counting. Data were analyzed from three independent vaginal tissues.

T-test was used to assess the differences between the study and control groups. Statistical analysis was performed using SPSS PC Version 9.0. $P < 0.05$ was considered to be statistically significant.

Results

The osmolality of OTC vaginal lubricants was measured using a vapor pressure 5520 osmometer (Table 1). All OTC lubricants showed high viscosity; they were therefore diluted 1/10-fold. Surprisingly, product no. 2 showed an osmolality of $5,275 \pm 25$ mmol/kg, while the osmolality for product no. 5 was not determined, as its osmolality exceeded the optimal range of the osmometer. Products no. 1, 3, and 4 showed an osmolality of 275 ± 25 mmol/kg, $1,800 \pm 32$ mmol/kg, and $1,233 \pm 11$ mmol/kg, respectively. Among all products tested, only one product was within the minimum WHO-recommended standards (< 380 mmol/kg) [3].

VK2/E6E7 cells were cultured with 5% dilutions of five OTC vaginal lubricants over 48 hours. As shown in Figure 1A, no treated control cells showed typical epithelial cell features (oval shape and plenty of cytoplasm). After treatment with the OTC vaginal lubricant products, all the treated cells underwent morphological changes, except for the cells treated with product no. 1. Over 50% of cells were detached from the culture dish plate and the remaining cells shrank into star-shaped structures. Among the cells treated with product no. 4, only 10-20% of the cells were detached and the cells showed less morphological changes than the cells treated with the other three products (no. 2, 3, and 5).

Table 1. — Osmolarity of the five OTC lubricants utilized in the study.

Product no.	Osmolality (mmol/kg)
1	275 ± 21
2	5,272 ± 76
3	1,800 ± 32
4	1,233 ± 11
5	ND*

To measure cell viability quantitatively, the authors performed the MTT assay and immunoblotting analysis. After treatment with OTC vaginal lubricants over 48 hours, they compared cell viability with the viability in the untreated controls (revised 100% viability). As shown in Figure 1B, the cells treated with products no. 2, 3, and 5 showed significantly decreased cell viability ($33.1 \pm 7.6\%$, $49.3 \pm 11.5\%$, and $37.8 \pm 5\%$, $p < 0.005$). The cells treated with products no. 1 and 4 showed decreased viability, but the difference was not statistically significant ($80.9 \pm 5.3\%$, $77.7\% \pm 9.8\%$, $p > 0.005$) compared to the controls. These data were confirmed through Western blotting; as seen in

Figure 2, the apoptosis or cell death-related molecules increased in the cells that were treated with the OTC vaginal lubricants having hyper-osmolality. The cells treated with product no. 3 did not express cleaved PARP. Occludin is an important tight junction-associated protein [11]. Expression of occludin protein was increased in all the cells treated with the lubricants, while the untreated controls showed no expression of the occludin molecule.

Five OTC vaginal lubricants were placed on top of the mucosal side of human vaginal tissues and cultured for 48 hours. As shown in Figure 3A, the untreated control showed intact and thick epithelial areas on the upper side of the stromal region, with high expression of bcl-2. The tissues treated with product no. 1 showed that the outer mucosal layer had partially vanished, while the bcl-2 level was high, the same as in the untreated controls. The remaining four tissue samples showed vanishing and damage of the epithelial and mucosal layers. The expression levels of bcl-2 were determined using bcl-2 positive cells in three different microscopic fields. The untreated controls and the tissues treated with product no. 1 showed higher expres-

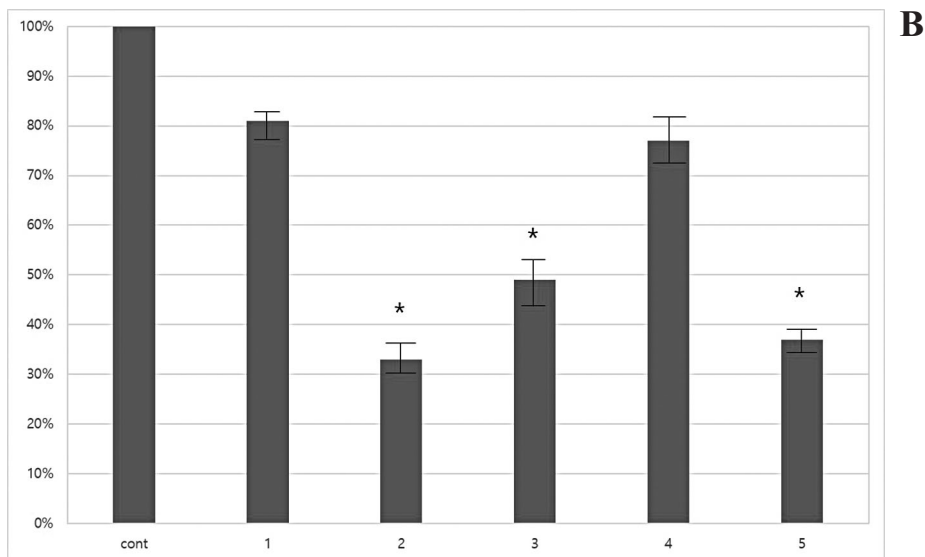
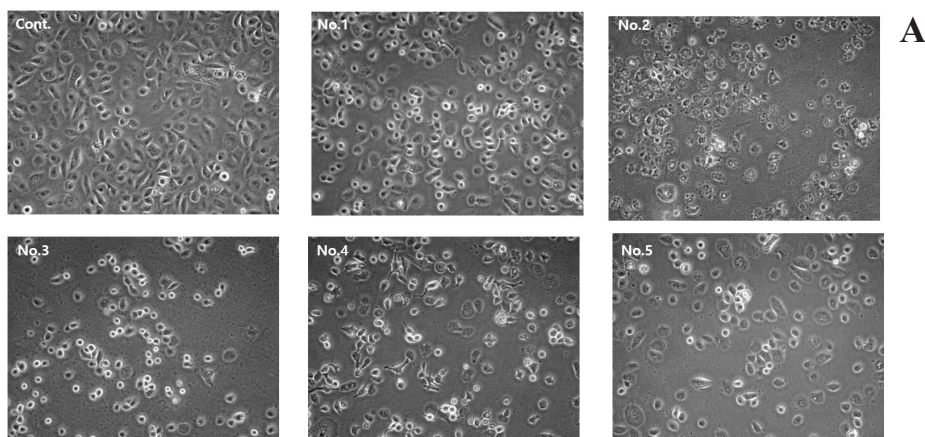


Figure 1. — Effect of OTC vaginal lubricants on the cultured human vaginal cell lines. (A) VK2/E6E7 cells are cultured with 5% dilutions of five OTC vaginal lubricants over 48 hours ($\times 200$). (B) Effect of OTC vaginal lubricants on viability of the cultured human vaginal cell lines. The data of bars are presented as mean \pm SD. * represents $p < 0.05$, compared with control.

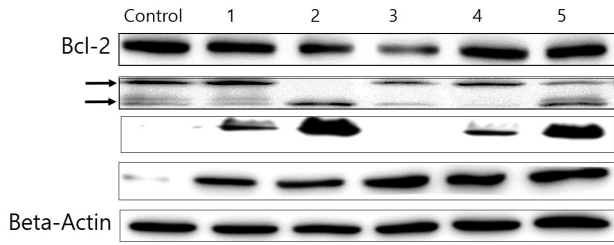
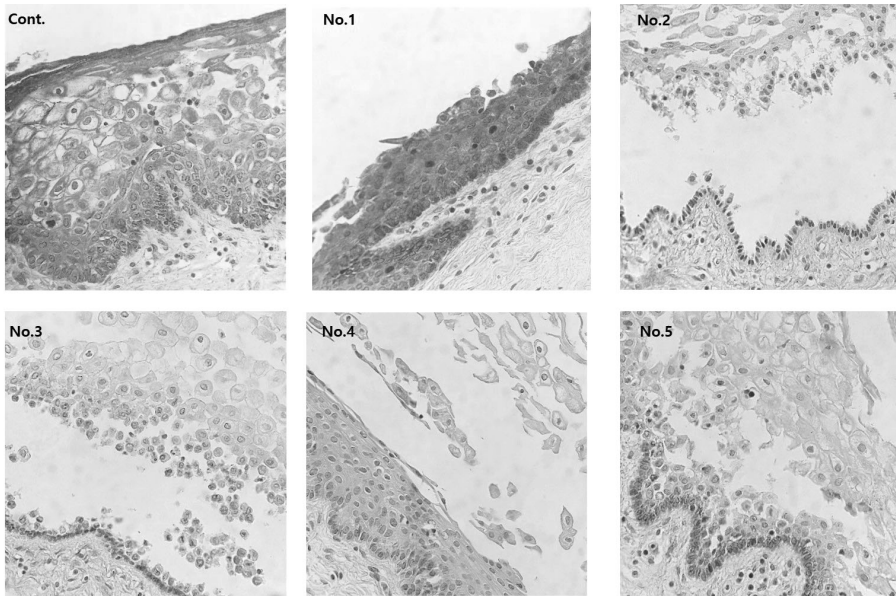
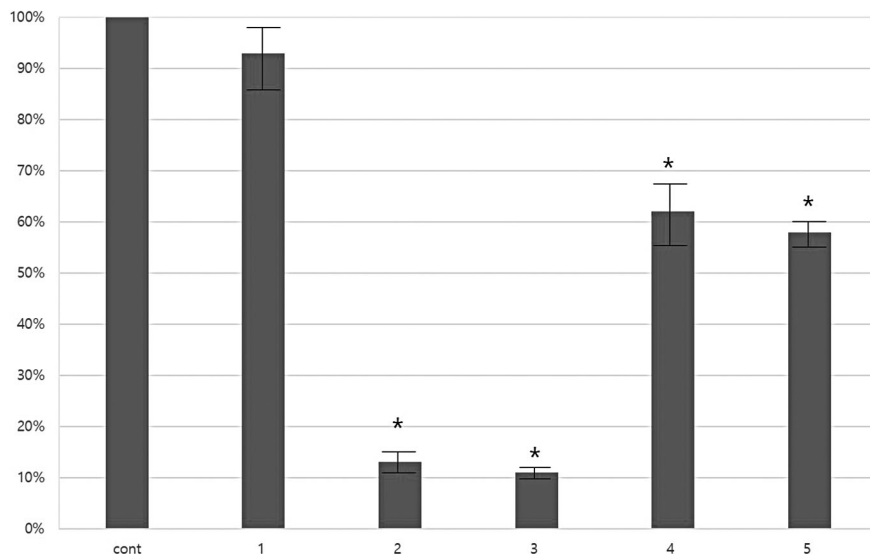


Figure 2. — Western blotting analysis for expression of Bcl-2, caspase 7, cleaved PARP, and occludin in VK2/E6E7 cells after 48-hour treatment of five OTC vaginal lubricants. β -actin serves as an internal control. A single band for each antibody is developed.

sion of bcl-2 (100% and 93.7 ± 11.2)(Figure 3B). The tissues treated with products no. 2, 3, 4, and 5 showed lower expression of bcl-2 than the untreated controls ($12.4 \pm 4.2\%$, $10.7 \pm 2.4\%$, $61.4 \pm 11.3\%$, and $59.2 \pm 5.4\%$ respectively, $p < 0.05$). Positive expression of cleaved caspase 3 was detected in all tissues, including the untreated controls (Figure 4A and B; $13.7 \pm 8.2\%$). Except for the tissues treated with product no. 1 ($18.9 \pm 3.5\%$), the tissues treated with the remaining four OTC lubricants showed significantly increased cleaved caspase 3 expression compared to the untreated controls (no. 2, $57.1 \pm 13.2\%$; no. 3, $31.2 \pm 2.1\%$; no. 4, $47.3 \pm 7.1\%$; no. 5, $78.1 \pm 14.2\%$, $p < 0.05$).

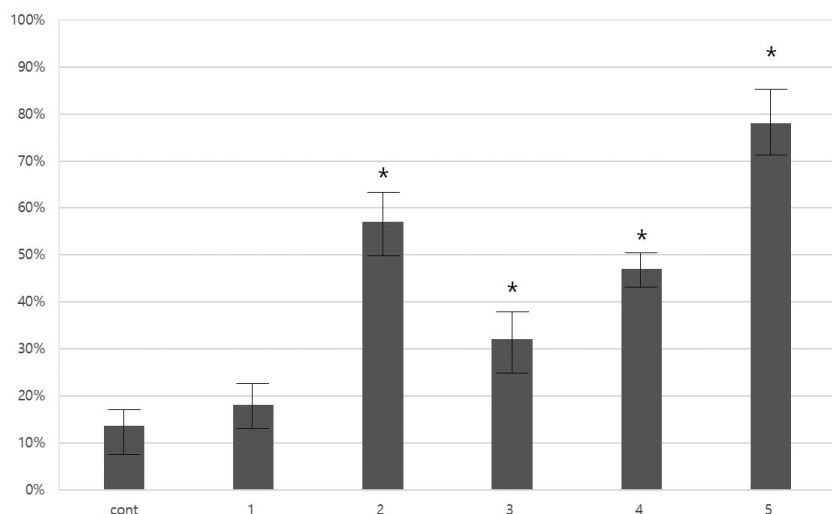
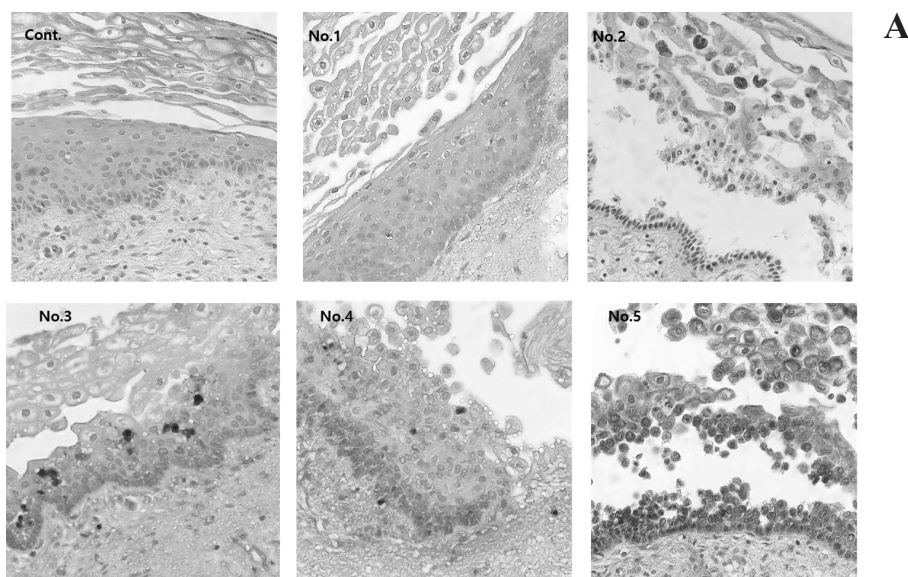


A



B

Figure 3. — Histological sections of vagina after 48 hours of treatment with different OTC vaginal lubricants. (A) Ultrastructural histological sections for expression of bcl-2 on human vaginal tissues ($\times 200$). The brown colored cells represent positive expression of bcl-2. (B) Expression of bcl-2 is analyzed by number of cells with brown-stained/total number of cells under the microscopic images ($\times 200$). Weak brown stains are excluded from the count. The data of bars are presented as mean \pm SD. * represents $p < 0.05$, compared with control.



B Figure 4. — Histological sections of vagina after 48 hours of treatment with different OTC vaginal lubricants. (A) Ultrastructural histological sections for expression of cleaved caspase 3 on human vaginal tissues ($\times 200$). The brown colored cells represent positive expression of cleaved caspase 3. (B) Expression of cleaved caspase 3 is analyzed by number of cells with brown-stained/total number of cells under the microscopic images ($\times 200$). Weak brown stains are excluded from the count. The data of bars are presented as mean \pm SD. * represents $p < 0.05$, compared with control.

Discussion

Vaginal lubricants intended for use during sexual activity are readily available for purchase in drug stores, through large retail chains, and over the internet. Women use vaginal lubricants to reduce vaginal dryness. Available pre-clinical and clinical data support that hyperosmolar vaginal products may be related to safety issues, and may also have detrimental effects on sperm motility, viability, and chromatin quality [1].

The present findings showed that four of the five OTC vaginal lubricants tested were hyperosmolar and resulted in cellular damage to the cultured vaginal cell line and human vaginal explant. One iso-osmolar OTC vaginal lubricant had less effect on both cultured vaginal cell lines and human vaginal explants.

Addition of OTC vaginal lubricants immediately induced morphological changes in the cultured vaginal cell line. The cell cytoplasm shrank and some cells showed rough surfaces. After 48 hours of treatment, cell death was seen in approximately 50% of the cells in the hyper-osmolar OTC lubricant-treated group. The cells treated with the iso-osmolar OTC vaginal lubricant showed fewer morphological changes and almost 80% of the cells survived with the same duration of treatment. Cellular viability was also measured 48 hours after OTC vaginal lubricant treatment. The cells treated with hyper-osmolar OTC vaginal lubricant showed decreased viability. To clarify the effect of OTC vaginal lubricants on cell death, the authors measured the expression of apoptosis-related proteins by the cells. The anti-apoptotic protein bcl-2 was detected in all the experimental conditions. The cells treated with products no. 2, 3, and 4

showed decreased expression of bcl-2, while the bcl-2 expression in the cells treated with products no. 1 and 5 was unchanged. The authors also measured the expression of other apoptosis-related proteins such as caspase-7 and cleaved PARP. In the case of caspase-7, the cleaved form was detected in the controls and in the cells treated with products no. 1, 2, 3, and 5. The cleaved form of PARP was detected in the cells treated with products no. 1, 2, 4, and 5. No. 1 and 4, which had relatively lower osmolality than the others, showed decreased level of expression of the cleaved form of PARP. The cleaved form of PARP was not detected in the cells treated with product no. 3 even when cleaved caspase 7 was detected in the same cells, making the data seemingly ambiguous. PARP is known to have the function of DNA repair and mediates apoptosis or necrosis of cells. Twelve DNA damage can induce an apoptosis initiation event that is mediated by caspase-3 activation and PARP cleavage. However, significant damage to the DNA repairing capacity resulted in cell necrosis without PARP cleavage.¹³ Therefore, the authors suggested that the OTC vaginal lubricants, regardless of their osmolality, could induce cellular change and hyper-osmolalones could even bring about cellular damage such as apoptosis or necrosis.

Occludin is a protein that is encoded by the OCLN gene in humans [14]. It is a 65-kDa (522-amino acid polypeptide) integral plasma-membrane protein located at the tight junctions [15]. In the present experimental condition, all OTC vaginal lubricant treated cells showed expression of occludin. It has already been mentioned in several studies that expression of occludin increases to protect cellular integrity and epithelial restitution under stressful conditions [16, 17].

As shown in Figures 3 and 4, treatment with OTC vaginal lubricants affected mucosal epithelial layers of the human vaginal tissue explants regardless of their osmolality. Product no. 1 that had iso-osmolality showed less epithelial dysfunction. Bcl-2 was highly expressed in the untreated control tissues and the tissue treated with product no. 1, but the mucosal epithelial layer was partially separated from its epithelium in the tissue treated with product no. 1 (Figure 3A). Minimum expression of cleaved caspase-3 was seen in the untreated controls and the tissues treated with product no. 1. These data showed that the osmolality of OTC vaginal lubricant is a major factor affecting epithelial cell survival and tissue integrity.

Dezzutti *et al.* reported that hyper-osmolality of OTC vaginal lubricants was associated with cellular toxicity and could potentially lead to increased susceptibility to HIV-1 and other sexually transmitted diseases [8]. Indeed, persons who consistently use hyper-osmolal lubricants showed a higher incidence of sexually transmitted diseases. In the present study, the extent of cellular or tissue damage did not correspond to the osmolality of the lubricants. There are different ingredients in every OTC vaginal lubricant, the major ingredients being water, glycerin, and other mois-

turizers. Some lubricants have *aloe barbadensis* leaf juice, alcohols, and natural flavors as additional ingredients. Especially, aloe vera is known to have a healing effect on skin wounds [18, 19]. However, these ingredients may bring about discordance between the osmolality and cellular toxicity. One of the OTC vaginal lubricants showed iso-osmolality. However, despite the physiological osmolality, it showed minimum cytotoxicity on the mucosal epithelium of vaginal tissue.

In summary, OTC vaginal lubricants are very frequently used by individuals who find intercourse inconvenient due to dryness and vaginal atrophy. In the present study, the authors were concerned with the high osmolality of OTC vaginal lubricants and evaluated their effects on the vaginal mucosal layer. The hyper-osmolality of OTC vaginal lubricants is a major cause of dysfunction of the vaginal mucosal layer. Therefore, the authors suggest that frequent use hyper-osmolal OTC vaginal lubricants may actually have an aggravating effect on vaginal dryness and infection. One of the limitations of this study is that it did not include all the OTC vaginal lubricants available in Korea; additionally, the effect of each ingredient contained in these products was not considered. Thus, additional research is needed to evaluate formulae to enable production of safer OTC vaginal lubricants.

References

- [1] Cunha A.R., Machado R.M., Palmeira-de-Oliveira A., Martinez-de-Oliveira J., das Neves J., Palmeira-de-Oliveira R.: "Characterization of commercially available vaginal lubricants: a safety perspective". *Pharmaceutics*. 2014, 6, 530.
- [2] Jamieson D.J., Steege J.F.: "The prevalence of dysmenorrhea, dyspareunia, pelvic pain, and irritable bowel syndrome in primary care practices". *Obstet Gynecol.*, 1996, 87, 55.
- [3] Edwards D., Panay N.: "Treating vulvovaginal atrophy/genitourinary syndrome of menopause: how important is vaginal lubricant and moisturizer composition?" *Climacteric*, 2016, 19, 151.
- [4] Herbenick D., Reece M., Sanders S.A., Dodge B., Ghassemi A., Fortenberry J.D.: "Women's vibrator use in sexual partnerships: results from a nationally representative survey in the United States". *J. Sex. Marital Ther.*, 2010, 36, 49.
- [5] Bakhtiari A., Basirat Z., Nasiri-Amiri F.: "Sexual Dysfunction in Women Undergoing Fertility Treatment in Iran: Prevalence and Associated Risk Factors". *J. Reprod. Infertil.*, 2016, 17, 26.
- [6] Keye W.R. Jr.: "Psychosexual responses to infertility". *Clin. Obstet. Gynecol.*, 1984, 27, 760.
- [7] Adriaens E., Remon J.P.: "Mucosal irritation potential of personal lubricants relates to product osmolality as detected by the slug mucosal irritation assay". *Sex. Transm. Dis.*, 2008, 35, 512.
- [8] Dezzutti C.S., Brown E.R., Moncla B., Russo J., Cost M., Wang L., *et al.*: "Is wetter better? An evaluation of over-the-counter personal lubricants for safety and anti-HIV-1 activity". *PLoS One*, 2012, 7, e48328.
- [9] Introini A., Vanpouille C., Grivel J.C., Margolis L.: "An ex vivo Model of HIV-1 Infection in Human Lymphoid Tissue and Cervico-vaginal Tissue". *Bio Protoc.*, 2014, 4, pii: e1047.
- [10] Rubin N.H., Baur P.S.: "A sample-grouping technique for paraffin embedments". *Stain Technol.*, 1983, 58, 157.
- [11] Penrose H.M., Marchelletta R.R., Krishnan M., McCole D.F.: "Spermidine stimulates T cell protein-tyrosine phosphatase-mediated protection of intestinal epithelial barrier function". *J. Biol. Chem.*, 2013,

- 288, 32651.
- [12] Herceg Z., Wang Z.Q.: "Functions of poly(ADP-ribose) polymerase (PARP) in DNA repair, genomic integrity and cell death". *Mutat. Res.*, 2001, 477, 97.
- [13] Agarwal A., Mahfouz R.Z., Sharma R.K., Sarkar O., Mangrola D., Mathur P.P.: "Potential biological role of poly (ADP-ribose) polymerase (PARP) in male gametes". *Reprod. Biol. Endocrinol.*, 2009, 7, 143.
- [14] Ando-Akatsuka Y., Saitou M., Hirase T., Kishi M., Sakakibara A., Itoh M., *et al.*: "Interspecies diversity of the occludin sequence: cDNA cloning of human, mouse, dog, and rat-kangaroo homologues". *J. Cell Biol.*, 1996, 133, 43.
- [15] Furuse M., Hirase T., Itoh M., Nagafuchi A., Yonemura S., Tsukita S., Tsukita S.: "Occludin: a novel integral membrane protein localizing at tight junctions". *J. Cell. Biol.*, 1993, 123, 1777.
- [16] He L., Zhou X., Huang N., Li H., Cui Z., Tian J., *et al.*: "Administration of alpha-ketoglutarate improves epithelial restitution under stress injury in early-weaning piglets". *Oncotarget.*, 2017, 8, 91965.
- [17] Li X., Liu X., Yu S.: "Psychological Stress-Derived Prolactin Modulates Occludin Expression in Vaginal Epithelial Cells to Compromise Barrier Function". *Cell. Physiol. Biochem.*, 2015, 37, 153.
- [18] Negahdari S., Galehdari H., Kesmati M., Rezaie A., Shariati G.: "Wound Healing Activity of Extracts and Formulations of Aloe vera, Henna, Adiantum capillus-veneris, and Myrrh on Mouse Dermal Fibroblast Cells". *Int. J. Prev. Med.*, 2017, 8, 18.
- [19] Galehdari H., Negahdari S., Kesmati M., Rezaie A., Shariati G.: "Effect of the herbal mixture composed of Aloe Vera, Henna, Adiantum capillus-veneris, and Myrrha on wound healing in streptozotocin-induced diabetic rats". *BMC Complement. Altern. Med.*, 2016, 16, 386.

Corresponding Author:
CHAN-WOO PARK, M.D.
Department of Obstetrics and Gynecology
Cheil General Hospital & Women's Healthcare Center
College of Medicine, Dankook University
17 Seoae-ro 1-gil
Jung-gu, Seoul 04619 (Korea)
e-mail: chanwoopark0805@gmail.com