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Ultrastructural Evaluation Following Catheterization of the Fallopian Tube with a Hysteroscopic Catheter

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Purpose: Our purpose was to assess the morphology and ultrastructural changes in the tubal epithelium following catheterization of the fallopian tube.

Methods: Fallopian tubes were obtained from 20 women who had undergone hysterectomies. Catheterization was performed in 20 tubes using a catheter developed for hysteroscopic tubal embryo transfer. The catheter has a 3-French diameter, tapering to 2 French (0.66 mm) at the tip portion. The 20 contralateral tubes served as controls and were not catheterized. Ultrastructural changes were examined by scanning electron microscopy and transmission electron microscopy.

Results: Scanning electron microscopy showed no transformation or defects of the tubal epithelium surface in catheterized or control tubes. Transmission electron microscopy showed no significant differences in the percentage of abnormal desmosomes and the percentage of basement membrane in ciliated and nonciliated cells between catheterized and noncatheterized tubes. No transformation or defects were observed in catheterized or noncatheterized tubes.

Conclusions: These findings suggest that catheterization of the tube using a hysteroscopic catheter caused no acute damage to the tubal epithelium.

KEY WORDS: catheterization; fallopian tube; scanning electron microscopy; transmission electron microscopy.

INTRODUCTION

Although the techniques of gamete intrafallopian transfer and zygote intrafallopian transfer are commonly

used in infertile women (1–3) and the procedure for cannulation used in correcting tubal obstruction has been improved (4,5), the effect of cannulation on the fallopian tube has rarely been examined. Because the diameter of the narrowest part of the tube is 0.4 mm (6) and the diameter of catheters for tubal catheterization is generally greater than 0.6 mm (7,8), it is possible that catheterization damages the fallopian tube. We used light microscopy, scanning electron microscopy (SEM), and transmission electron microscopy (TEM) to examine the in vitro effects of catheterization on the fallopian tube using a catheter with a smaller diameter that was recently developed for tubal cannulation with hysteroscopy. We investigated the morphological characteristics of the basement membrane profile, the desmosome profile, and the number of mitochondria. These organelles are labile, and changes in their appearance are sensitive indicators of environmental disturbances.

MATERIALS AND METHODS

Patients

Fallopian tubes were obtained from 20 premenopausal women who underwent total abdominal hysterectomies at Keio University Hospital because of uterine myoma or adenomyosis. Tubes were obtained in the follicular phase in seven women, at midcycle in six women, and in the secretory phase in seven women. The fallopian tubes were macroscopically normal. Informed consent was obtained from all women prior to surgery.

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Fig. 1. The tip of the new Teflon catheter for hysteroscopic tubal embryo transfer is 3 cm long. The catheter diameter is 3 French, tapering to 2 French (0.66 mm) at the tip portion. The tip is marked off in 5-mm segments.

Catheter Specification

We used a new Teflon catheter developed in cooperation with Olympus (Tokyo). The catheter, which has a diameter of 3 French, tapering to 2 French (0.66 mm) at the tip, is inserted into the fallopian tube with a hysteroscope. The tip of the catheter is 3 cm long and marked off in 5 mm. The catheter is flexible and requires no guidewire for insertion (Fig. 1).

Surgery

The catheter was introduced through the operating channel of the hysteroscope (HYF Type-P, Olympus, Tokyo). The patient was placed in the lithotomy position and a speculum was inserted into the vagina. The hysteroscope was inserted via the cervical canal into the uterine cavity using carbon dioxide for distention before hysterectomy was performed under anesthesia. After the tubal ostium was visualized, the catheter was introduced into the tube. The contralateral tube was not catheterized and served as the control. The catheter was advanced 3 cm into the tube, and carbon dioxide insufflation was terminated. After approximately 1 min, the hysteroscope with the catheter was removed. Immediately after the uterus and tubes had been gently excised, the uterus was cut with scissors from the cervix to the tubal cornu. The fallopian tube was dissected 3 cm from the ostium, and 5×5 -mm cross sections were obtained from the tube at 5-mm intervals using a scalpel.

Fixation and Preparation for Electron Microscopy and Light Microscopy

Samples were fixed by immersion in 2% glutaraldehyde at 4°C for 2 hr, followed by overnight fixation in 0.1 M cacodylate buffer at 4°C.

SEM

Samples were dehydrated in increasing concentrations of isopropanol (50, 60, 70, 80, 90, and 95%), dried in liquid CO₂, and etched under a dissecting microscope. Specimens were mounted on aluminium stubs with the epithelial side upward, coated with gold, and observed in a Hitachi SV-4000 at an operating voltage of 15 kV. We examined 32 tubes by SEM, including 16 catheterized tubes and 16 noncatheterized tubes, obtained from 16 patients (mean age, 46.3 ± 4.8 years).

TEM

Fallopian tubes ($n = 8$) were fixed in 2% glutaraldehyde and 0.1 M cacodylate buffer for SEM and post-fixed with 1% osmium tetroxide at 4°C for 1.5 hr. Specimens were dehydrated in the same ascending alcohol concentrations as in the SEM preparation procedure. Sections were then stained with uranyl acetate and embedded in an Epon 812 mixture. Areas of epithelial cells in the fallopian tube were identified in semithin sections stained with toluidine blue. Thin sections were obtained from the embedded Epon sections and mounted on 200-mesh copper grids. Grids stained with gold were observed in a Hitachi HU-12AS TEM at an operating voltage of 75 kV. TEM was used to examine desmosome profile, basement membrane characteristics and mitochondria of cell organelles in four catheterized and four noncatheterized tubes. The disappearance of desmosomes was determined in 20 ciliated cells and 20 other cells per sample. Basement membrane characteristics were examined in 20 sections with and 20 sections without catheterization. In addition, mitochondria were counted in 20 ciliated cells and 20 other cells (e.g., secretory cells) per sample.

Light Microscopy

The semithin toluidine blue-stained sections used for TEM were also observed by light microscopy to determine the extent of damage to ciliated cells.

The microscopist was unaware of the identity of the study group until after all specimens had been assessed.

Statistics

Statistical analysis was performed by the chi-square test with Yates correction. A *P* level of less than 0.05 was considered statistically significant.

Fig. 2. Scanning electron micrograph of the luminal surface of a noncatheterized fallopian tube. (A) A large number of ciliated cells and secretory cells can be observed. (B) Ciliated cells are intermingled with microvilli. CC, ciliated cell; MV, microvilli; S, secretory cell. Original magnifications: (A) $\times 4500$; (B) $\times 7000$.

RESULTS

SEM

Many ciliated cells and secretory cells were observed in tubes without catheterization (Fig. 2A). Mainly ciliated cells intermingled with microvilli were observed in the lumen of the oviduct (Fig. 2B). Many ciliated cells were also observed in catheterized tubes; the ciliated cells showed no evidence of damage (Fig. 3A). The ciliated cells and the microvilli on the dome-shaped secretory cells were intact, showing no evidence of damage (Fig. 3B). The surface of the tubal lumen exhibited no transformation after catheterization, and no defects in the ciliated cells or secretory cells were observed in any phase of the menstrual cycle.

TEM

The tubal epithelium was composed mainly of nonciliated cells, with occasional ciliated cells (Figs. 4A and 5A). The nonciliated cells were covered with numerous microvilli and filled with abundant secretory granules. Occasional granules and other cytoplasmic organelles were also released into the lumen from the superficial cytoplasm. Various cytoplasmic organelles, including mitochondria, free ribosomes, rough endoplasmic reticulum, and Golgi areas, were observed (Figs. 4B and 5B). The cilia and microvilli showed no evidence of damage in tubes with and without catheterization.

Desmosomes, zona attachment, and basement membranes were mechanisms of cell attachment in the extracellular area. Abnormal desmosomes in ciliated cells were observed in 4 (10%) of 40 sections in catheterized tubes.

Fig. 3. Scanning electron micrograph of the luminal surface of a catheterized fallopian tube. (A) Many ciliated cells can be observed and show no evidence of damage. (B) Ciliated cells and microvilli on dome-shaped secretory cells are intact. CC, ciliated cell; S, secretory cell; MV, microvilli. Original magnifications: (A) $\times 1800$; (B) $\times 4600$.

Fig. 4. (A) Transmission electron micrograph of the tubal epithelium in a catheterized tube shows mainly nonciliated cells, with occasional ciliated cells. (B) Cytoplasmic organelles, including mitochondria and rough endoplasmic reticulum, can be observed in the tubal epithelium. In the extracellular area, desmosomes and the basement membrane are visible. NCC, nonciliated cell; CC, ciliated cell; M, mitochondria; RER, rough endoplasmic reticulum; D, desmosome; BM, basement membrane. Original magnifications: (A) $\times 1500$; (B) $\times 3000$.

terized tubes and in 1 (2.5%) of 40 sections in noncatheterized tubes. In nonciliated cells, abnormal desmosomes were observed in one section (2.5%) with and without catheterization, respectively. No basement membrane deformities were observed in tubes with or without catheterization. We identified 1012 mitochondria in 80 ciliated cells of four catheterized tubes and 1196 mitochondria in 80 ciliated cells of four noncatheterized tubes. The mean number of mitochondria per ciliated cell was 12.6 ± 6.4 in catheterized tubes and 15.0 ± 4.9 in noncatheterized tubes. We counted 978 mitochondria in 80 nonciliated cells of four catheterized tubes and 1134 mitochondria in four noncatheterized tubes. The mean number of mitochondria per cell was 12.2 ± 5.0 in catheterized tubes and 14.2 ± 7.3

in noncatheterized tubes. These differences were not significant.

Light Microscopy

Light microscopy showed no defects or deformities in the epithelium of the tubal lumen in catheterized tubes (Figs. 6 and 7). Ciliated cells were observed in tubes with and without catheterization. Secretory granules were observed in nonciliated cells in tubes with and without catheterization.

DISCUSSION

The ampullary-isthmus junction of the fallopian tube, which extends 3–5 cm from the uterus, is the

Fig. 5. (A) Transmission electron micrograph of the tubal epithelium in a noncatheterized tube; the basement membrane is normal. (B) The tubal epithelium shows no defects of the cilia. BM, basement membrane; C, cilia; D, desmosome; M, mitochondria; RER, rough endoplasmic reticulum. Original magnifications: (A) $\times 1800$; (B) $\times 3000$.

normal site of fertilization and early embryonic development in vivo. Although catheters with diameters of approximately 3 French (1 mm) are usually used for transvaginal tubal catheterization (5,7,9), these catheters are inserted only 0.5–1 cm from the tubal ostium. We have developed a new catheter with a 3-cm tip

Fig. 6. Toluidine blue-stained semithin section of a tubal lumen in a noncatheterized tube. The epithelium shows no changes. C, cilia. Original magnification, $\times 400$.

Fig. 7. Toluidine blue-stained semithin section of the tubal lumen in a catheterized tube. Clumps of cilia are visible. C, cilia; Original magnification, $\times 400$.

that improves the transfer of embryos to the ampulla. This catheter, which tapers from a 3-cm tip with a 2-French (0.66-mm) diameter, can reach the isthmus portion. The diameter of the tip portion is smaller, because the catheter requires no guidewire for insertion. The diameter of the narrowest part of the fallopian

tube, in particular, the interstitial, intramural, and isthmus portions, is 0.4 mm (6). Thus, with its 0.66-mm outer diameter, the tip of our newly developed catheter could press on the tubal tissue.

An elevated intraluminal pressure may lead to morphological changes, such as a loss of mucosal folds, atrophy of the epithelium, and deciliation of various degrees (10). The percentage of ciliated cells in the fimbria has been found to be decreased significantly during and after salpingitis (11). Abnormalities of the fallopian tube seen in women with overt and silent pelvic inflammatory disease include flattened mucosal folds, extensive deciliation, and degeneration of the secretory epithelial cells (9). Large areas of the luminal surface of the mucosa of blocked fallopian tubes lack both the secretory and the ciliated cells (12). SEM showed no transformation or defects in ciliated cells and other cells on the surface of the tubal lumen after catheterization in the present study. Because the tubal epithelium is thrown into folds, it is difficult that most of the surface of the tubal lumen is observed in the SEM study. TEM showed no damage on the surface of the tubal lumen after catheterization. Thus, insertion of the new catheter appeared to exert little pressure on the surface of the tubal lumen and the resulting luminal distention caused no apparent ultrastructural changes. The proportions of ciliated cells and nonciliated cells vary during the menstrual cycle (11). We observed no transformation or defects of the surface of the tubal lumen after catheterization in any phase of the cycle, suggesting that the new catheter caused little damage to the tubal lumen.

The new catheter rubs the tubal lumen, and thus, catheterization could potentially damage the structure of the tube. The disappearance of desmosomes is a characteristic of cell degeneration (13). There was no significant difference in the percentage of desmosomes in ciliated cells and other cells in tubes with and without catheterization in the present study. These results indicate that insertion of the new catheter did not cause cell degeneration in the tubal lumen and had little effect on desmosomes of ciliated and other cells. We did not evaluate desmosomes in every phase of the menstrual cycle. Crow *et al.* (14) observed no significant variation in the number of some cytoplasmic organelles at different stages of the menstrual cycle. Thus, examination of the number of desmosomes in different phases of the menstrual cycle should be adequate.

The thickening and destruction of the basement membrane suggest that the basal cell layer responds to trauma with metabolic hyperactivity, and, moreover,

damage to the basement membrane should be considered as the mechanism of trauma to the epithelium, such as in the vocal cord (15). We observed no deformities of the basement membrane in fallopian tubes with or without catheterization in the present study, suggesting that insertion of the new catheter caused little damage to the epithelium of the tubal lumen.

Mitochondria are highly sensitive to adverse environmental influences, so that changes in their morphology may provide an early indication of environmental disturbances (16,17). TEM showed no significant differences in the mean number of mitochondria in ciliated and nonciliated cells in tubes with and without catheterization in the present study, suggesting that catheterization of the tube had no adverse effects on the environment. The smooth endoplasmic reticulum is one of the most sensitive indicators of injury (18). Fallopian tubes with or without catheterization showed no deformities in the smooth endoplasmic reticulum or other cytoplasmic organelles, indicating that insertion of the new catheter caused few morphological changes in the tubal epithelium and did not disturb the intratubal environment.

The status of the fallopian tube affects the quality of mammalian embryos (19–21). Major midcycle proteins in the human oviduct may protect the biochemical milieu of the fallopian tube during early embryonic development (22). Fertilization of oocytes could be affected. The catheter used in the present study caused no ultrastructural changes, if the intratubal milieu were altered by catheter insertion, indicating that it had little effect on the intratubal milieu.

We evaluated structural changes in the tubal tissue immediately after catheterization. Although catheterization may have long-term effects, analysis of long-term effects of catheterization on the tubal structure is technically difficult. However, Gleicher *et al.* (4) found that the pregnancy rate in patients who underwent transcervical balloon tuboplasty increased up to 12 months after the procedure, suggesting that catheterization may not have subacute or chronic adverse effects on the intratubal milieu.

In conclusion, the present ultrastructural observations provide evidence that the use of the new catheter for transcervical tubal catheterization had little effect on the tubal epithelium or the intratubal milieu.

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