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Transmission Electron Microscopy: Evaluation of Damage in Human Oviducts Caused by Different Surgical Instruments

AUGUST C. OLIVAR, M.D.*
FARIPOUR A. FOROUHAR, M.D.**
CONCETTINA G. GILLIES, M.S.**
and DAVID R. SERVANSKI, B.A.**

*Hartford Hospital**
Hartford, CT

*University of Connecticut Health Center***
Farmington, CT

ABSTRACT

Different wave length lasers (CO2, Nd-YAG, KTP-532), electrocautery and radiofrequency instruments were used to assess the degree of tissue damage in human oviducts.

Excision of the human fallopian tube for tuboplasty is achieved most often by electrocautery, scalpel and, more recently, the Carbon Dioxide (CO2) Laser. There is not enough evidence at the present time to scientifically justify using one particular mode of incision which would show minimal damage to surrounding healthy tissue, especially heat lateral damage.

The present study compares the tissue damage produced by the microelectrocautery, the CO2, the KTP-532, the Nd-YAG lasers and the radiofrequency surgical instrument, utilizing different power densities on the fallopian tubes freshly removed at the isthmic portion, taken from healthy women ages 30–42. Transmission electron microscopy sections at the cellular level show that the electrosurgical radiofrequency surgical instrument produces the least damage to surrounding healthy tissue. The CO2 laser with intermittent superpulse mode showed the second lowest amount of damage. The most damage was observed with the Nd-YAG laser at high power densities.

Introduction

When hemostasis and cutting are desired, the most common instrument used is the electrocautery; however, the degree of lateral thermal damage produced by this instrument is often suspected to be extensive. For this reason other instruments, such as the carbon dioxide[1–4] as well as other lasers (Argon, KTP-532, Nd-YAG), have been recently introduced, with the hope that they would produce less damage to surrounding tissues.

Incisions of fallopian tubes for relatively common operations, such as ectopic pregnancies or tuboplasties, are achieved most often with the scalpel, electrocautery or CO2 laser; however, there is not enough evidence to sci-

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entifically justify one particular mode of incision which would produce minimal or no damage to surrounding healthy tissue and still be able to produce adequate hemostasis.

Following the principles of microsurgery, more adhesion formation and compromised organ function are expected with more mechanical injury. It would be logical to think that in choosing the best instrument to incise an organ (in this case the oviduct), it should be the one producing the least lateral and depth thermal damage. Moreover, the improvement of surgical techniques should enhance reproductive performance and possible better pregnancy success rates.

The present study was designed to obtain information on different types of "cutting" instruments which could be useful for clinical applications, knowing the vast amount of reproductive surgical procedures performed on the oviducts via laparoscopy or laparotomy.

Materials and Methods

Sections of the isthmic portion of oviducts were obtained from premenopausal patients (30–42 years of age) undergoing abdominal or vaginal hysterectomies with bilateral salpingooophorectomies for benign indications, other than pelvic inflammatory disease. The study was approved by the Institutional Research Committee.

Specimens obtained were immediately inspected by the pathologist and allowed to be used if no gross abnormalities were seen. Incisions on the oviducts were performed at a speed of 1.4 to 1.6 cm per second. The isthmic portion of the oviduct was then incised using the following instruments: Electrocautery (Valley Laboratories, Inc., Boulder, CO) with a microelectrode of approximately 0.12 mm diameter at 3 and 5 settings (approximately 110 volts at 500 ohm load ± 20 percent) 0.4 Mhz frequency 726; Radiofrequency Surgical Instrument (Ellman International, Inc., Hewlett, NY) with microelectrode of approximately 0.12 mm diameter at 3 and 5 settings (110) volts at 400 ohm load), giving approximately 90.75 and 151.25 watts, 4.0 Mhz frequency; CO2 laser (Sharplan Lasers, Inc., Allendale, NY) at 5, 25 and 50 watts settings with 0.2 mm spot diameter (power densities of approximately 12,500, 62,500 and 125,000 watts/cm^2, respectively) Continuous Mode (CM) and Intermittent Super Pulse (ISP) mode at an average of 15 watts; KTP-532 (Laserscope, Santa Clara, CA) at 5, 15, 20 watts, with a 0.6 mm fiber (1,388, 4,166 and 5,555 watts/cm^2), Continuous Mode; Nd-YAG laser (Laserscope) at 5, 25, and 50 watts with a 0.6 mm fiber (1,388, 6,944 and 13,888 watts/cm^2), Continuous Mode.

The following formulas were used to calculate approximate power densities: a) for the electrocautery and radiosurgical units:

$$\text{Power (watts)} = \frac{(\text{voltage})^2 \times \text{setting}}{\text{load resistance}}$$

b) for the lasers:

$$\text{P.D.} = \frac{\text{watts} \times 100}{(\text{spot diameter})^2 \text{ in mm}} \text{ watts/cm}^2$$

These two formulas can give approximate power densities. Formula (b) could loosely be applied to calculate power densities in electrocoagulation and radiosurgery; however, in applying either formula one must take into consideration that they are only gross approximate calculations. Samples taken from the incision area were immediately placed in cacodylate buffered 4 percent glutaraldehyde for 4–6 hours, changed to 0.1 M cacodylate buffer and refrigerated. Each specimen was then examined under a dissecting microscope and divided into thirds for subsequent processing. After dehydration through a graded series of alcohols, propylene oxide and infiltration with a resin, the pieces were embedded in flat molds. Each piece was oriented so that the extent of tissue damage could be assessed through longitudinal sections.

Thick, 1 micron sections were cut and stained with toluidine blue. Thin, 60 nm sections were then cut and stained with Uranylacetate and lead citrate, and examined in a Philips EM 300. Each section was picked up on a copper, thin bar grid measuring 3.05 mm

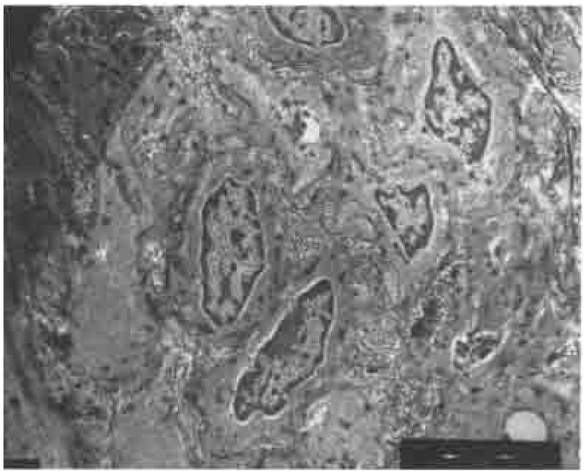

FIGURE 1. Transmission electron micrograph (×14,000) showing well defined, healthy cellular structures at 0.3 mm section, using two radiofrequency surgical instruments.

diameter. The grid space measures 115 μm and the bar measures 10 μm. (fig. 1) Tissue damage was evaluated by identifying the treated surface and looking at the cells in each square in sequence. Cellular damage was noted by changes indicating cell death, i.e. swollen mitochondria, nuclear changes in chromatin pattern and loss of cell architecture. The distance in "squares" from the surface was noted for each treatment.

TABLE I

Transmission Electron Microscopy Showing Lateral Thermal Damage on Human Oviducts by Different Instruments

Instruments	*Power Densities (dial setting in watts or numbers)*	*Diameter of the Electrode or Spot in mm*	*Extent of Tissue Damage in mm*
Electrocautery	3	0.12	0.550
	5	0.12	0.750
Radiosurgery	3	0.12	0.3
	5	0.12	0.3
KTP–532 Laser	5	0.6	0.600
	15	0.6	0.800
	20	0.6	1.2
Nd–YAG Laser	5	0.6	0.800
	25	0.6	0.800
	50	0.6	1.3
CO2 Laser (CM)	5	0.25	0.600
	25	0.25	0.550
	50	0.25	0.5
CO2 Laser (ISP)	15[a]	0.25	0.350

CM = Continuous mode.
ISP = Intermittent super pulse.
[a]Average wattage.

Results

Table I summarizes the extension of heat lateral damage of the oviductal isthmic portions. When incisions were made with the microelectrocautery, tissue damage was observed up to 0.550 mm from the incision area.

Incisions made with the radiofrequency surgical instruments produced only 300 μm of damage, regardless of the dial setting (or power densities). The KTP-532 and the Nd-YAG lasers, at high power densities, produced the most heat lateral damage. The CO2 laser and the KTP-532, at intermediate power densities used in a Continuous Mode, produced damages up to 500 and 600 μm, respectively; however, the CO2 laser, using an average of 15 watts with an Intermittent Super Pulse (ISP) Mode, only produced up to 350 μm of heat lateral damage. Examples of these damages can be seen in fig. 1, showing a section of the oviduct at 0.3 mm from the impact area, with well defined, morphologically undamaged cellular structures, utilizing the radiofrequency surgical instrument. Fig. 2, in contrast, showed damaged cellular structures at 1.2 mm from the impact area when high power densities (13,888 watts/cm^2) were used with the Nd-YAG laser. Fig. 3 shows almost undamaged cellular structures at 0.3 mm sections when Intermittent Super Pulse mode was used with the CO2 laser. Sections at 0.35 mm demonstrated normal cellular structures.

Discussion

Previous studies by Baggish and Chong,[1] utilizing Scanning Electron Microscopy (SEM) in human oviducts with the CO2 laser, showed the heat lateral damage to be up to 1

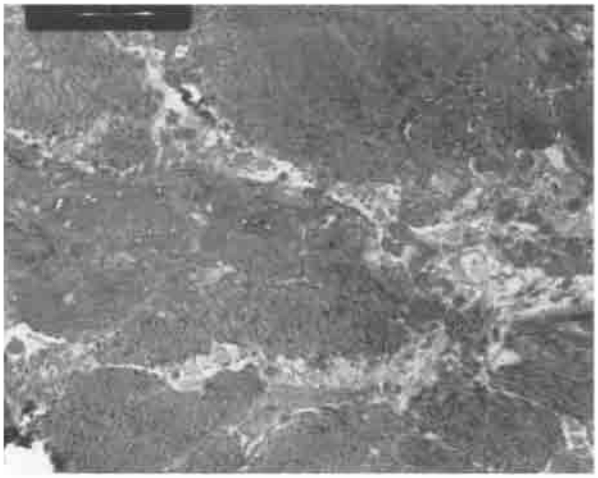

FIGURE 2. Transmission electron micrograph (×17,000) of the oviduct at 1.2 mm section, showing damaged cellular structuring by the Nd-YAG laser.

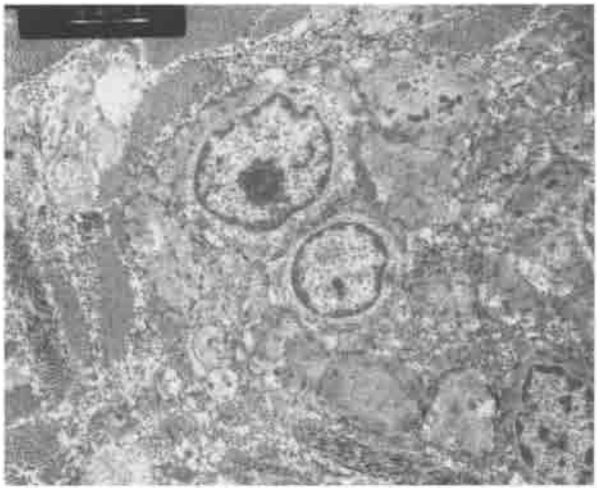

FIGURE 3. Transmission electron micrograph (×14,000) of a section of the oviduct at 0.3 mm with the CO2 in a superpulse mode, showing almost healthy cellular structures.

mm lateral to the impact zone; however, the power densities in that study were lower (700 watts/cm^2), bigger spot diameters were utilized and SEM only showed surface damage (1.5 mm). Later on, studies by Bellina,[6] comparing electrocautery incisions versus CO2 laser in the rabbit, showed less tissue injury with the CO2 laser. Tissue damage was assessed by light microscopy, which does not produce enough magnification or adequate resolution to assess tissue damage at the cellular level.

Luciano et al.,[7] showed that the depth of thermal injury on either the ovarian or uterine tissue was similar with the CO2 laser or the microelectrocautery, as was the postoperative adhesion formation in the rabbit model.

In this study the Nd-YAG laser was utilized using the 600 mm "naked" fiber at high power densities (approximately 41,600 W/cm^2) and the KTP-532 (at 16,600 W/cm^2). It was observed that the tissue experienced the most heat lateral damage, more than when lower power densities were used.

This observation is different from that of the CO2 laser, which produces less heat lateral damage when high power densities are used, especially with the Intermittent Super Pulse Mode as noted by Baggish et al.,[8] which is corroborated in this study. For the KTP-532 laser, the best results are found at intermediate power densities, between 1400 and 2800 watts/cm^2 (5 to 10 watts with 0.6 mm spot diameter). Electrocautery instruments, which operate between 0.4 and 1.5 Mhz (400,000 to 1.5 million cycles per second), produce intermediate heat lateral damage. The radio frequency surgical instrument, however, was observed to produce the least heat lateral damage of all instruments used (table 1). This phenomenon is probably due to the much higher cycle frequency in which this instrument operates (3.8 to 4.0 Mhz or 3.8 to 4 million cycles per second). Since the diameter of the cutting instrument or the size of the spot diameter of the laser seems to play a role in some cases on the extension of the heat lateral damage, it seems logical to think that if the diameter of the microelectrode, fiber or spot, is smaller, the radius of heat extension, frequency or radiation may also be smaller. Clinically it would make sense, therefore, to use the smallest possible diameter one could obtain when incisions are made, but that is not the only parameter one should consider when trying to determine "the best instrument," as demonstrated in the comparison in table I.

The findings in this study could be applied clinically to select the most appropriate instrument for incision proposed for the fallopian tubes, especially in reproductive surgery with the hope of improving reproductive performance.

Summary

To determine the extent of lateral thermal damage at the cellular level, Scanning (SEM) and Transmission Electron Microscopy (TEM) of sections of human oviducts were taken after incisions were performed, utilizing electromicrocautery, radio-microsurgery, carbon dioxide (CO2) laser, KTP-532 and Nd-YAG lasers at different power densities. Incisions (vaporization) with the Nd-YAG and the KTP-532 fiber lasers at high power densities produced the most thermal damage. Intermediate thermal damage was observed when the KTP-532 laser was used at intermediate power densities, as well as with the microelectrocautery and the CO2 laser at high power densities. The least amount of thermal damage was observed when incisions were made with the radiosurgical instrument.

These observations could be applied clinically, and considered when choosing the best instrument to perform conservative surgery of the fallopian tubes.

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