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Blockade of tubal patency following transcervical administration of polidocanol foam: initial studies in rhesus macaques

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Abstract

Objective—To demonstrate the feasibility of polidocanol foam (PF) as a nonsurgical method of female permanent contraception using a nonhuman primate model.

Study Design—Four groups of adult female rhesus macaques underwent either transcervical treatment with 5% PF directly into the uterine cavity, treatment with inert (methylcellulose, MF) foam or no treatment followed by removal of the reproductive tract for histologic evaluation. Untreated animals were included in Group 1 (n= 3). Group 2 animals (n= 4) were treated once with MF. Group 3 (n=7) received a single, and Group 4 (n= 5) received multiple monthly treatments with PF; in these 2 groups, baseline tubal patency was assessed either laparoscopically by chromopertubation (CP) or by hysterosalpingography.

Results—Group 1 (untreated) and Group 2 (MF) animals had normal tubal histology. In contrast, Group 3 and 4 females treated with PF showed evidence of tubal damage. In Group 4, bilateral tubal blockade was noted on CP after two (n=2) or three (n=3) treatments. Histologic analysis confirmed complete tubal occlusion (loss of epithelium, fibrosis) in 3 of these animals and 1 showed significant tubal damage localized to the intramural segment. Nontarget (cervix, vagina, endometrium, ovary) reproductive tissues were unaffected. While similar tubal changes were observed after a single treatment (Group 3), endometrial hemorrhage was also noted

Conclusion—PF is a promising candidate agent for nonsurgical permanent female contraception. The histologic features of PF occlusion are confined to the intramural portion of the tube.

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Implications—This study in rhesus macaques supports further development of transcervical administration of polidocanol foam as a nonsurgical approach to permanent contraception. A nonsurgical method could reduce risks and costs associated with surgical female sterilization, and increase access to permanent contraception.

Keywords

tubal occlusion; nonhuman primate; sterilization; nonsurgical; female

1. Introduction

Globally, surgical sterilization is the most common and effective method of birth control [1]. Lack of access to highly effective reversible modern methods does not explain the popularity of permanent methods. In the United States, female sterilization is the most prevalent contraceptive method, reported by 24% of married and 21% of all women, in the most recent data from the National Survey of Family Growth [2]. Unfortunately, in many lesser-developed nations the availability of this method is limited by a scarcity of providers and surgical facilities, and by concerns for surgical risks [3]. Recent guidelines from World Health Organization (WHO), the US Agency International Development (USAID) and the Bill and Melinda Gates foundation underscore the need to develop new nonsurgical approaches to female sterilization [4].

The transcervical administration of active agents directly into the uterine cavity represents one approach to nonsurgical permanent contraception. Quinacrine, tetracycline, doxycycline, erythromycin, chloroquine, demeclocine, primaquine, and trimethoprim have been evaluated using the rat uterine and other animal models for their potential ability to cause tubal sclerosis and occlusion [5-8]. Quinacrine is the best-characterized agent, with multiple human studies completed including large clinical trials [9-12]. Questions regarding potential toxicity of an approach known as *Quinacrine Sterilization* (QS) emerged after widespread human use was initiated in many developing nations [13, 14]. Since QS clinical trials occurred in the absence of regulatory approval of QS for use in the United States or Europe, these allegations led to a loss of confidence in the approach, and withdrawal of support by governments and funding agencies. Subsequently completed epidemiologic studies [15, 16] support the safety of QS. However, serious concerns regarding implementation of the program and a significantly higher (2-4 times greater than surgical tubal ligation at 10 years) failure rate [17, 18] present significant obstacles to further research with this agent.

Investigation of alternative agents for transcervical, non-surgical female permanent contraception is warranted. One potential candidate agent is polidocanol (hydroxy-polyethoxy-dodecane), a synthetic long-chain fatty alcohol originally developed and marketed as an injectable local anesthetic [19]. It was first used in Germany as a sclerosing agent for the cosmetic treatment of veins in the 1960s, and was quickly adopted for that use in many European countries. In 2010, 0.5% and 1% polidocanol solutions received FDA approval in the United States for the treatment of uncomplicated “spider” and “reticular” varicose veins up to 3mm in diameter. The drug has been intensely studied and is extremely

well characterized with a high therapeutic index of safety; hematoma, irritation, discoloration and pain related to the injection site are the most frequent adverse effects [19].

Since polidocanol is a registered drug for sclerosis of veins, approval of this treatment for permanent contraception could proceed much faster than for a new chemical entity not yet been cleared by the FDA. Preliminary studies of transcervical administration of 1% polidocanol solution in the rat uterine horn model [20] demonstrated uterine effects similar to those reported in animals treated with quinacrine or erythromycin in protocols that resulted in impaired fertility [8]. No systemic or peritoneal toxic effects of polidocanol were noted in the treated rats. However, when 1% polidocanol solution was administered transcervically to rhesus monkeys, the expected outcomes of fibrosis and tubal occlusion were not observed [20]. While it is possible that the primate reproductive tract is not susceptible to polidocanol in the same manner as the rat, other explanations such as drug concentration, length of tissue exposure to the drug, and number of treatments have all been shown to be critical variables in the use of quinacrine and erythromycin for non-surgical sterilization [8, 21, 22]. Studies on increasing the efficacy of polidocanol during sclerotherapy have concluded that introduction of the agent as foam rather than a liquid greatly increases the efficacy for vein occlusion [23-25]. The scientific basis of this approach is that the foam state allows greater surface concentration of polidocanol at the point of contact with the epithelium without increasing the overall dose. While the side-effect profile of polidocanol foam is similar to liquid, visual disturbances and migraine are more frequent and stroke has been reported [26].

The complex cervical anatomy of the rhesus macaque cervix [27], presents a significant challenge to the development of a macaque model for transcervical procedures. In this study we refined methods to catheterize the macaque cervix. We then conducted experiments to test the hypothesis that the transcervical administration of polidocanol foam could block the fallopian tubes without adverse nontarget effects in the reproductive tract.

2. Methods

2.1 Animal Care

A total of 22 adult female rhesus monkeys (*Macaca mullata*) between the ages of 8-19 years were used in this study. Animal husbandry provided by Oregon National Primate Research Center (ONPRC), West Campus of Oregon Health & Science University (OHSU) is in accord with the National Institutes of Health (NIH) Guidelines for Care and Use of Laboratory Animals [28]. The ONPRC Institutional Animal Care and Use Committee reviewed and approved all study protocols prior to initiation of the research. Stage of the menstrual cycle was determined by measurement of serum estradiol (E2) and progesterone (P) by the Endocrine Services Core Laboratory, ONPRC. E2 and P assay was carried out with a specific electrochemoluminescent Roche Elecsys 2010 analyzer (F. Hoffmann-La Roche Ltd, Basel Switzerland).

2.2 Surgical procedures

We used several approaches to introduce a small catheter or spinal needle into the uterine cavity for assessment of tubal patency or PF administration. The procedures were carried out under sterile technique and when possible during the follicular phase of a menstrual cycle. The animals were sedated with ketamine, received prophylactic antibiotics (Cefazolin, 30 mg/kg), and then underwent laparoscopy or ultrasound to assist with the placement of a catheter or needle into the uterine cavity for assessment of tubal patency and/or delivery of the foam treatment. The ectocervix was visualized in the upper vagina with a small Pederson speculum that had been modified to reduce its length to 3 cm. To introduce a catheter, the cervix was stabilized with a forceps applied to the anterior or posterior lip and then a 1 mm cervical dilator was used to identify the path under laparoscopic or ultrasound guidance. Once the path was identified, a 1.5 mm dilator was threaded through a Soules Intrauterine Insemination catheter (model J-IUIO-532500, Cook, Bloomington, IN) modified to reduce its overall length fitted such that the dilator served as an obturator. When the dilator tip entered the uterine cavity, the catheter was advanced over the rod into the uterus, and the rod was removed. An alternative approach was to insert a 1-mm stainless steel dilating rod and a nested set of sequentially-sized ridged cannula. Once positioned through the internal os under laparoscopic or ultrasound guidance, this rod was used as an obturator for the introduction of the smallest cylinder. A series of these small hollow cannulas (1.2-3 mm outer diameter) were available in a nested set, such that it was possible to insert a larger cannula over a smaller cannula to dilate the cervix atraumatically. The 3 mm cannula was of sufficient diameter to permit the passage of a small silicone HSG balloon catheter (model J-CHSG-503000, Cook, Bloomington, IN) into the uterine cavity. The cannula was then withdrawn, and the balloon inflated with 0.3 to 0.5 mL of saline to facilitate anterograde delivery of solution through the uterus. A third approach was developed because simple catheterization was not successful in all animals. In this approach we accessed the uterine cavity by directly passing an 18 gage 3.5 inch spinal needle (model 405184, BD, Franklin Lakes, NJ) through the cervical colliculum directly into the uterine cavity under ultrasound guidance. Foam or contrast media were delivered directly through the needle.

Laparoscopy was performed under general anesthesia using a 5-mm umbilical port for the laparoscope and a 5-mm suprapubic port for a manipulating probe. Once a catheter was secured in the uterine cavity, tubal patency was verified by chromopertubation using a dilute solution of indigo carmine in saline instilled transcervically through the catheter. Patency was confirmed laparoscopically by observing spill of dye from the right and left tubal fimbria

For ultrasound procedures, a transabdominal transducer was used to visualize the cervix and uterine cavity and guide the placement of instruments. A hysterosalpingogram (HSG) was performed by infusing contrast media (Isovue-370, Bracco Diagnostics, Princeton, NJ) through the uterine catheter or needle. A scout film was exposed to verify correct positioning followed by a second exposure after delivery of 1.5 to 2 mL of contrast media. If bilateral patency was not observed, repeat exposures were performed up to a maximum of 10 mL of contrast or 5 total films.

2.3 Foam infusion

A 5% solution of polidocanol was prepared by mixing 10 mL of polidocanol stock (Sigma P9641) with 190 mL of sterile saline. An inert (control) foam solution was created by dissolving 300 mg methylcellulose (Sigma #7140) in 30 mL sterile saline. Foam was created using the two syringe technique originally described by Cavezzi and Tessari [29]. A total of 2mL of polidocanol or methylcellulose solution was thoroughly mixed over 60 – 90 seconds with 8 mL of air until 10mL of foam of uniform consistency was prepared. A control-tip syringe containing the foam was then attached via a Luer lock connection to the infusion catheter or needle, and the foam was instilled into the uterine cavity. The presence or absence of spill of foam from the tubal fimbria on each side was recorded by video laparoscopy. Visualization of foam movement into the tubes was not possible for animals examined with ultrasound only. Initially, the foam was administered only until spill was observed from the tubal fimbria (a total of 5-10 mL of foam). As we gained more experience with the procedure, some animals received multiple instillations of foam such that the entire pelvis was filled (40-150 ml delivered) to intentionally maximize the exposure of the pelvic organs to the possibility of nontarget adverse effects of treatment on the pelvic peritoneum. However, it was not possible to quantify the amount of foam delivered to the peritoneal cavity. For most procedures, additional foam was infused until uterine pressure led to backflow of foam through the cervix or until 30 mL of solution (150mL foam) was administered. Instruments were then removed and the procedure was terminated. Animals received standard post-operative care, and were returned to standard husbandry within 24 hours.

2.4 Experimental Groups

Animals were allocated into groups based on exposure to the foam treatment (e.g. no foam (untreated), inert foam, or PF multiple or single treatment). A baseline clinical evaluation of tubal patency was performed when possible. Because of restrictions on the number of animal procedures, not all animals could undergo a repeat surgical procedure prior to necropsy. Some animals early in the study did not have an ex vivo evaluation of patency. Characteristics of the groups are as follows:

Group 1, untreated animals—The animals in this group did not receive any intrauterine treatments prior to necropsy. Some of these animals failed an attempted transcervical procedure. Information on baseline tubal patency was not available and the cases are presented to provide representative control histology only.

Group 2, Methylcellulose foam single treatment—All but one of the females in this treatment group received transcervical methylcellulose foam under ultrasound guidance without a baseline assessment of tubal patency followed by an intramuscular injection of depomedroxyprogesterone acetate (DMPA, 3.5 mg/kg mg). They were re-examined clinically 4 weeks later immediately prior to necropsy. One animal in this group underwent a baseline HSG that showed tubal occlusion; she was not treated with DMPA, and her necropsy was done at one week. Animals in this group came from the same pool of animals used in Group 3. DMPA was tested as a strategy to improve the ease of transcervical catheter placement.

Group 3, 5% polidocanol foam single treatment—Following an initial exam and laparoscopic evaluation documenting unilateral or bilateral tubal patency, females in this treatment group received transcervical 5% PF as described above and were then scheduled for necropsy 1 or 2 weeks later without retreatment or a clinical assessment of tubal patency. Animals in this group differed from Group 4, as they were available only for short term projects; necropsy was indicated for clinical reasons unrelated to the protocol.

Group 4, 5% polidocanol foam multiple treatment—Following an initial exam and laparoscopic evaluation documenting unilateral or bilateral tubal patency, females in this treatment group received transcervical 5% PF as described above. Tubal patency was re-assessed at approximately 30 day intervals using the same techniques outlined above. After insertion of the laparoscope, the peritoneal cavity was inspected, and any new findings were noted and documented with photographs. If dye spill was observed from one or both tubes, the polidocanol foam infusion was repeated. If no dye spill was observed from either tube, the animal was euthanized and the reproductive tract collected for analysis. In the event that tubal patency was observed at the 3rd laparoscopy, the animal was re-treated and scheduled for necropsy after 30 additional days of observation.

2.5 Pathologic examination and Histology

A standard gross post mortem evaluation was performed for all animals by veterinary pathologists at the ONPRC Pathology Services Unit according to their standard necropsy protocol. In all cases, the pelvic organs were visualized through a midline incision, and photographs were obtained of any gross abnormalities. The uterus and cervix, upper vagina, bilateral tubes and ovaries were extirpated as a single specimen and placed in a physiologic saline solution (Hank's Balanced Salt Solution; Sigma). For four of the animals that received multiple treatments with 5% PF, non-target tissues (liver, kidney, spleen, lung, etc) were evaluated microscopically. Tissues were fixed in 10% neutral buffered formalin, processed with standard histologic techniques, cut at 5 microns and stained with hematoxylin and eosin. Blood samples for laboratory chemistries, hematologic profiles and hormone levels were obtained at baseline, and at the end of treatment.

In each case, the extirpated reproductive tract was inspected for gross anatomical abnormalities. An HSG catheter was placed transcervically in specimens from most of the foam-treated animals and indigo carmine or India ink was instilled (ex vivo) transcervically to assess tubal patency. Further dissection was performed under a stereomicroscope to free the fallopian tube from the mesosalpinx, broad ligament and ovary. Representative tissue blocks were prepared from the distal fimbria, ampullary and isthmic sections of the tube. In addition, the uterine cornua containing the interstitial portion of the tube, was arranged so that the entire segment from uterine serosa to the endometrial cavity would be available in a single block. Additional specimens were obtained for evaluation of the endometrium, myometrium, endocervix, ectocervix, vagina, and ovaries. Tissues were fixed in 4% buffered paraformaldehyde, embedded in paraffin, sectioned and stained with hematoxylin-eosin following standard methods (Sigma-Aldrich). Immunohistochemistry for oviductal glycoprotein was performed with polyclonal anti-OVGP IgG (ProSCI, Poway CA; cat #4763) following methodology described previously [30].

2.6 Data analysis

The study was designed as a descriptive project to demonstrate proof of the concept that polidocanol foam can occlude the primate tube. The primary outcome is histologic evidence of tubal occlusion. The number of animals selected was considered the minimum needed to establish the technique, and to exclude biologic variability as a factor in treatment outcome. Descriptive summary statistics are reported for each group.

3. Results

A total of 22 animals were evaluated in the study. A transcervical procedure was attempted in 20; two that did not undergo any procedure are included as untreated controls. Attempts at placement of a transcervical catheter failed in 6/20 (30%). Placement of a transcervical needle was successful after failed catheter placement in two cases where this was attempted. Four other animals did not undergo an attempt at the transcervical needle procedure; three of these animals were released from the protocol and excluded from the results as histologic samples were not obtained, and the other animal is included as an untreated control.

3.1 Clinical evaluation of tubal patency

A baseline assessment of tubal patency was performed by either CP or HSG in 13 animals. In this group, most showed evidence of tubal patency (bilateral (8/13, 62%) or unilateral (1/13, 8%)), but 13% (3/13) had occlusion of both tubes on the baseline study (Table 1). A fundal perforation occurred in one animal on the initial assessment, so patency could not be assessed. Bilateral patency was confirmed on the second laparoscopy.

Baseline evaluation of tubal patency was performed in only one of the animals treated with methylcellulose foam (Group 2) and in none of the untreated controls (Group 1). Seven animals received a single treatment with PF (Group 3), followed by necropsy at 2 weeks (n=3), 1 week (n=3) or immediately (n=1) after exposure. Two animals in this group had baseline bilateral tubal occlusion; both had associated chronic health problems (severe pelvic endometriosis, chronic-active colitis). None of the Group 3 animals showed clinical evidence of bilateral occlusion following a single PF treatment.

All of the Group 4 animals (n=5) had normal anatomy and underwent successful transcervical placement of an intrauterine catheter followed by CP to document baseline tubal patency, followed by multiple treatments with 5% PF. Bilateral patency was noted on the initial surgery in three animals, and unilateral patency in one; patency from the blocked tube of this animal was noted at a subsequent surgery. During instrumentation of the cervix, fundal perforation occurred in one animal such that baseline patency could not be assessed; bilateral patency was observed on the next evaluation. None of these animals demonstrated any adverse effects of treatment. Although all five Group 4 animals showed clinical evidence of tubal patency by CP in at least one tube one month following the initial treatment, bilateral tubal occlusion was observed after two (n = 2) or three (n = 3) PF treatments (Table 1).

3.2 Gross and histologic findings

No significant abnormalities were seen in the fallopian tubes of untreated controls (Group 1) or in animals exposed to inert methylcellulose foam (Group 2). However, a baseline evaluation of tubal patency was performed in only one of these animals (Table 1). One of the Group 3 animals had severe pelvic endometriosis and a large submucous myoma. There were no gross abnormalities of the reproductive tract in any of the other animals examined. Omental adhesions to the ovary were noted in one animal as a baseline surgical finding. These are common in animals from our colony that have undergone previous surgery. One Group 4 animal experienced a fundal uterine perforation during treatment and developed an omental adhesion at that site along with other mild peritoneal adhesions. No other significant bowel or pelvic adhesions developed during treatment.

Representative samples from the fallopian tubes, uterus, cervix, and vagina were evaluated. Among animals treated with PF, there were no gross or microscopic abnormalities noted in the fimbria, ampulla, or isthmus of the tube. In contrast, there was a significant loss of epithelium in the intramural portion of the tube in most of the polidocanol foam-treated animals that became complete in some regions, and was associated with a hyalinized amorphous area in the former muscular subepithelial zone not seen in untreated, or methylcellulose-foam treated controls (Figure 1). In successive sections, the lumen became obliterated. Prior to reaching the endometrium, the lumen re-opened, and ultimately became re-epithelialized. Oviductal glycoprotein (OGP), is an estrogen regulated glycoprotein expressed in the fallopian tube but not in the myometrium [30]. We performed immunohistochemistry and detected OGP in the areas of abnormal histology, confirming that these distorted regions were part of the fallopian tube (data not shown). There were no histologic abnormalities noted in the vagina, cervix, or endometrium of treated animals (Fig. 2).

All but one of the five Group 3 animals with baseline tubal patency showed the typical histologic changes of de-epithelialization and hyalinization confined to the intramural tube. The exception was an animal thought to have had inadequate treatment (< 10 mL foam administered) due to high intrauterine pressure. Both of the Group 3 animals with baseline occlusion received PF treatment in the uterine cavity; the histology of the tubes was normal in one, and not assessed in the other. In addition to the observed tubal changes, endometrial hemorrhage confined to the functional layer was observed in Group 3 animals evaluated 1 and 2 weeks after exposure to PF. There were no abnormalities noted in other reproductive tissues.

Among animals that received multiple PF treatments (Group 4), these typical histologic features were noted in three of the five animals (Table 2). The intramural tube appeared normal in one animal treated 3 times with PF; this animal had been noted to have bilateral obstruction by CP and on ex vivo examination using India ink in the laboratory. Of note, this animal received treatment with PF during the luteal phase of 3 cycles; all of the other females were treated in the follicular phase. A second animal showed the typical histologic features of occlusion and blockade on CP and with the India ink test, but a small amount of ink was noted in the tubal isthmus suggesting the possibility of patency. In general, the

degree of de-epithelialization, sub-epithelial hyalinization and luminal occlusion was more advanced in animals in Group 4.

3.3 Safety

All animals underwent complete gross and histopathologic evaluation of nontarget tissues. One of the animals in Group 4 showed evidence of chronic peritonitis felt to be secondary to an old bowel perforation or to the multiple laparoscopies. Segmental atherosclerotic changes in the iliac artery of uncertain etiology were noted in 3 of the five Group 4 animals. Mild proliferative change to the intima of the iliac artery was seen in 2/5 Group 3 animals with baseline patent tubes. One animal in Group 3 had severe pelvic endometriosis; this animal died during treatment with PF (see below). No other unusual findings were noted in nontarget tissues including the brain, heart, lungs, liver, and kidneys of any of the four animals from Group 4 that received an expanded tissue evaluation (data not shown). Similarly, there were no clinically important changes in the hematologic profile, or clinical chemistries compared to baseline data obtained from the same animals, and treatment did not affect menstrual cyclicity (data not shown).

There was one animal death associated with PF treatment. A 14 y female with heavy menstrual bleeding, thought to have endometriosis was assigned to Group 3. Abnormal anatomy was identified at laparoscopy with probable endometriosis and adhesions to the right adnexa, but the left tube appeared normal. Although a left cervical perforation occurred during dilation, it was possible to pass a dilator through the cervix into the uterine cavity, and an intrauterine balloon catheter was advanced without difficulty to the fundus. Tubal patency was not observed from either tube. Shortly after the PF instillation was begun, the left tube appeared dusky, and the animal began to experience respiratory and cardiac compromise. Despite attempts at resuscitation, the animal succumbed. At necropsy, numerous clots were noted in dilated veins including the common iliac veins, caudal vena cava, mesenteric veins, pulmonary veins, and in the right ventricle and atrium. The histologic and pathologic features were consistent with disseminated intravascular coagulation, although air embolism could not be ruled out. There were no other adverse effects of treatment noted in other animals.

4. Discussion

This study reports the first intrauterine application of PF in a nonhuman primate model. Our results demonstrate that 5% PF caused tubal obstruction in the cornual portion of the oviduct. In contrast, none of the untreated or methylcellulose foam-treated control animals, or animals with baseline clinical evidence of blocked tubes showed similar histologic changes. All of the animals evaluated in our small series that underwent multiple clinical assessments required more than one PF treatment to stimulate tubal occlusion. Bilateral tubal occlusion was not observed clinically in any of our Group 4 animals one month following initial treatment, and up to three treatments were needed to produce clinical evidence of bilateral blocked tubes in some animals.

Most of the animals treated with PF showed characteristic histologic changes of de-epithelialization and hyalinization confined to the intramural segment of the tube. These

histologic features are similar to those reported in cynomolgus monkeys [7] within one week of treatment with intrauterine quinacrine, and in women [31] 6 -20 weeks after treatment with intrauterine doses of quinacrine known to result in permanent contraception. Although the changes were similar following one, or multiple treatments, we did not observe animals for an extended period after a single treatment, so these experiments do not provide conclusive evidence that a multiple treatments or a longer time interval is required for occlusion to develop, or that a single treatment would be as effective as multiple treatments. However, one of the primary limitations with Quinacrine sterilization was the high failure associated with a single treatment [17]. This would suggest that over time, re-epithelialization may occur in the damaged region.

Some of the PF-treated animals that had clinical evidence of occlusion by the dye test also had histologic features of normal tubes. Although it is possible that a narrow area of occlusion was missed during preparation of slides, these likely represent treatment failure. Spasm at the utero-tubal junction may prevent adequate treatment of the fallopian tube. It is also possible that some animals may have an inherent resistance to the damage induced by PF treatment.

In a preliminary study we found that transcervical treatment of rhesus macaques with 1% polidocanol liquid using a similar protocol [20] failed to result in tubal occlusion. The histologic features of epithelial destruction and fibrosis observed with polidocanol foam seen in this study were not observed with polidocanol liquid. The properties of foam enhance the sclerosing effects of polidocanol liquid by effectively increasing the local concentration. In essence, the fine bubbles exclude the non-reactive water molecules such that the highly reactive hydrophilic portion of the long-chain fatty acid is in direct contact with the epithelial cells. Since foam is a transient state, this also increases safety, as the bubbles slowly dissipate resulting in a less reactive liquid. Therefore, it is not surprising that the maximal effect of polidocanol foam observed in our specimens is confined to the narrow intramural portion of the tube. The high pressure of bubbles moving across the epithelium in this small diameter tube likely produces a mechanical and chemical effect leading to deep epithelial damage. The pressure is reduced as the foam moves to the larger lumen of the isthmus and ampullary portions of the tube, becoming very low as it spills into the peritoneal cavity. Consider by analogy the cleaning action of a soap solution delivered by a power washer nozzle. The effect is maximal at the point of initial contact and much less impressive as the pressure reduces. Studies on increasing the efficacy of polidocanol during sclerotherapy have concluded that introduction of the agent as foam rather than a liquid greatly increases the efficacy of great saphenous vein occlusion [23-25].

The one treatment-related animal death due to air embolism or disseminated intravascular coagulation deserves special discussion. This animal had baseline tubal occlusion and a submucous myoma that prevented the balloon catheter from expelling as intrauterine pressure increased with treatment. As a consequence, a total of 30 mL of foam appeared to enter the uterine and ovarian veins. Each 10 mL of foam contains 8 mL of air. A recent review by Guex [26] details the complications and safety of polidocanol sclerotherapy when the agent is administered directly into veins. The complications consist primarily of thrombosis and gas embolism. Thrombosis occurs as a result of clot formation that occurs in the treated

veins. While our technique does not involve injection of PF directly into a vein, the possibility of inadvertent venous uptake during treatment is a concern. Gas embolism results from the gas used to make foam. Air is the most common gas used in clinical sclerotherapy practice. Gas embolism can result in clinical symptoms and events such as transient visual disturbance, migraine, stroke, or even death. Normally, the sclerosing properties of PF do not exist when bubbles exit the uterine cavity and enter the circulation. Studies performed with Varisolve™ (a polidocanol endovenous microfoam (PEM)) currently in Phase 3 clinical trials for the treatment of saphenofemoral junction incompetence) have shown that the bubbles do not contain any sclerosing properties once they reach the pulmonary capillary bed [26]. Significant intravascular uptake from intrauterine administration must be uncommon, as most of the monkeys treated with PF received high volumes (80 – 150 mL) of foam without any evidence of adverse effects. Although the small size of macaques (6-9 kg) likely magnifies the risk of PF treatment, strategies to prevent or minimize inadvertent intravascular uptake of PF must be developed to ensure safety prior to moving on to phase I trials in women. To improve safety and efficacy, we are currently investigating refinements in our approach. These include experiments to determine the optimal dose/concentration and rate of polidocanol administration, co-administration of accessory sclerosants (e.g. doxycycline), and use of CO₂ to generate foam.

Our experimental approach allowed us to observe the spill of polidocanol foam into the pelvic where it came into contact with the pelvic organs and bowel. While the procedures were performed under anesthesia, there was no evidence of unusual pain or any untoward events during post-operative recovery. These findings are consistent with the observed absence of any adverse effects of treatment on repeat laparoscopy or at necropsy, or upon detailed histopathologic evaluation of adjacent tissues and critical distant organs (e.g. brain, liver, kidney, heart). The general safety profile of polidocanol for intravascular injection has been established, and the compound is FDA approved for the treatment of spider veins and small reticular veins up to 3mm in diameter.

This study validates the use of the nonhuman primate (NHP) as an animal model for permanent contraception. Reproductive physiology and menstrual cyclicity in macaques is very similar to that of humans, making them an ideal model [32]. However, one limitation of the macaque model is that the cervical anatomy is complex, with a prominent colliculum that obstructs passage of instruments into the uterine cavity [27]. While we have achieved success in transcervical procedures in macaques with careful technique, many animals screened for our study were not suitable for experiments. The transcervical needle technique represents one approach to surmount this problem. Other nonhuman primate species such as the baboon have a straight cervical canal and may be more suitable for transcervical manipulations.

Our results demonstrate that a clinical evaluation of tubal patency with chromopertubation or by HSG may provide false evidence of tubal blockade when the tubes are in fact histologically normal. Furthermore, one of both tubes may appear blocked on one evaluation, and patent in a subsequent study. The precise etiology of “tubal spasm” is unknown, but a clinical diagnosis of tubal non-patency is more likely in the luteal phase [33]. If the tubal spasm is unilateral, successful blockade of the contralateral tube with PF

might result in an increase in intrauterine pressure such that on a subsequent exam the previously “closed” tube opens.

Our report of successful blockade of the fallopian tubes in macaques represents an initial step in a preclinical program evaluating polidocanol foam for permanent contraception. Widespread interest in the concept of a simple, safe nonsurgical approach to female sterilization led to the promotion and use of QS in many developing nations during the late 1980 and early 90s, including India, Vietnam, and Chile [10, 34-36]. While this extensive experience demonstrates that the concept of non-surgical sterilization is feasible and acceptable for human medicine, it also provides a cautionary story. The enthusiasm for and promotion of strategies for fertility control in developing countries that are not approved in the United States or European Union leads to suspicion regarding the safety of methods and the goals and motives of international family planning programs. Successful validation of a single treatment approach leading to high efficacy and rigorous evaluation of safety will be required prior to early phase clinical trials in women. The goal of human studies will be initial regulatory approval and clinical use in the United States prior to clinical trials in low resource settings.

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Figure 1.

Representative histologic changes from untreated control (A) rhesus macaques and following a single transcervical administration of methylcellulose (B) or one (C) and two (D) treatments with 5% polidocanol (C) foam. Cross sections through the cornual region of the uterus are shown. Note the loss of epithelium, subepithelial hyalinization, and luminal obliteration in the sections from polidocanol-treated animals.

Figure 2.

Representative histologic features of the vagina, cervix, and endometrium from untreated control rhesus macaques (upper panels) and from animals that underwent necropsy 4 weeks after receiving two or three treatments with 5% polidocanol foam (lower panels). The histology from methylcellulose-treated tissue was no different than control animals (not shown). Vagina (A & D); Cervix (B & E); Endometrium (C & F).

Table 1

Clinical evaluation of rhesus macaque tubal patency as assessed by laparoscopy (chromopertubation (CP)) or by hysterosalpingography (HSG). After extirpation of the reproductive tract at necropsy, patency was also assessed ex vivo by chromopertubation using India ink. Animals in Group 4 had multiple assessments and treatments with polidocanol foam, while Group 3 animals had only a single assessment and treatment. Only one of the Group 2 animals and none from Group 1 had a baseline assessment of tubal patency. DMPA = depomedroxyprogesterone acetate, NA = not assessed

animal	method	initial assessment		second assessment		third assessment		fourth assessment		necropsy	
		right	left	right	left	right	left	right	left	right	left
Group 1 Untreated controls											
22287#	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
23771	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
20981	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Group 2 Methylcellulose foam single treatment/DMPA											
23767	NA	NA	NA	NA	NA	NA	NA	NA	NA	patent	patent
28879	NA	NA	NA	NA	NA	NA	NA	NA	NA	patent	patent
30131	NA	NA	NA	NA	NA	NA	NA	NA	NA	patent	patent
21928 [^]	HSG	closed	closed	NA	NA	NA	NA	NA	NA	closed	closed
Group 3 5% polidocanol foam, single treatment											
21869	CP	patent	patent	NA	NA	NA	NA	NA	NA	patent	closed
25953	CP	patent	patent	NA	NA	NA	NA	NA	NA	NA	NA
24577	CP	closed	closed	NA	NA	NA	NA	NA	NA	NA	NA
20435	CP	closed	closed	NA	NA	NA	NA	NA	NA	NA	NA
21759	HSG	patent	patent	NA	NA	NA	NA	NA	NA	patent	patent
22208	HSG	patent	patent	NA	NA	NA	NA	NA	NA	patent	patent
25223	HSG	patent	patent	NA	NA	NA	NA	NA	NA	patent	patent
Group 4 5% polidocanol foam, multiple treatment											
29395	CP	patent	patent	patent	patent	closed	closed	NA	NA	closed	closed
27586 [*]	CP	patent	patent	NA	NA	patent	patent	patent	patent	closed	closed
27587	CP	patent	patent	patent	closed	patent	patent	NA	NA	closed	closed
27595	CP	closed	patent	closed	patent	patent	closed	closed	closed	closed	closed

animal	method	initial assessment		second assessment		third assessment		fourth assessment		necropsy	
		right	left	right	left	right	left	right	left	right	left
29421 ⁺	CP	unsure	unsure	patent	patent	closed	closed	NA	NA	closed	closed

* 27586 Failed cervical procedure during second assessment, no tubal assessment or PF treatment.

⁺ 29421 A fundal perforation occurred during the first laparoscopy. Patency confirmed with second scope

[^] 21928 Did not receive DMPA

[#] 22287 Failed attempted transcervical procedure

Table 2

Histologic evaluation of tubal patency. NE = normal epithelium, AE = abnormal epithelium, DE = complete de-epithelialization, H = sub-epithelial hyalinization, CO= complete luminal occlusion; PO= partial luminal occlusion, NO = normal lumen. The patency assessment was the final pathologic assessment based upon the histologic features

animal	# of treatments	timing of necropsy	histologic features	patency assessment
Group 1	Untreated controls			
22287	0		NE, NO	Open
23771	0		NE, NO	Open
20981	0		NE, NO	Open
Group 2	Methylcellulose foam single treatment/DMPA			
23767	1	4 weeks, DMPA-treated	NE, NO	Open
28879	1	4 weeks, DMPA-treated	NE, NO	Open
30131	1	4 weeks, DMPA-treated	NE, NO	Open
21928 [^]	1	one week	NE, NO	Open
Group 3	5% PF single treatment			
21869	1	2 week after 1st treatment	DE, H, CO	Closed
25953	1	2 week after 1st treatment	DE, H, CO	Closed
24577	1	2 week after 1st treatment	NE, NO	Open
20435	1	immediate	Not assessed	Not assessed
21759	1	1 week after 1st treatment	DE, H, CO	Closed
22208	1	1 week	NE, NO	Open
25223	1	1	DE, H, CO	Closed
Group 4	5 % PF multiple treatments			
29395	2	4 weeks after 2nd treatment	AE, H, CO	Closed
27586	3	4 weeks after 3rd treatment	NE, NO	Open
27587	3	4 weeks after 3rd treatment	DE, H, CO	Closed
27595	3	4 weeks after 3rd treatment	AE, PO	Equivocal (dye seen)*
29421	2	4 weeks after 2nd treatment	DE, H, CO	Closed

[^] 21928 Did not receive DMPA

* the microscopic presence of dye was observed in the absence of gross observation of dye spill ex vivo prior to tissue preparation.