Characterization of tubal occlusion after transcervical polidocanol foam (PF) infusion in baboons.

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Abstract

Objective: Our long term goal is to develop a nonsurgical method of fallopian tubal occlusion for the purpose of permanent contraception. We have previously demonstrated that transcervical administration of 5% polidocanol foam (PF) can create tubal occlusion in macaques, but that multiple treatments are required. In this study we assessed the efficacy of various regimens of PF with and without depo medroxyprogesterone [DMPA] (to control ovarian cycle phase) in the baboon.

Study Design: Adult cycling female baboons were evaluated for tubal patency by hysterosalpinography (HSG) and then received a transcervical infusion of PF with (+) or without (-) an intramuscular injection of DMPA (3.5mg/kg). Two concentrations of PF were compared: 1% [(+) DMPA, n=5; (-) DMPA, n=3] and 5% [(+) DMPA, n=4; (-) DMPA, n=3]. Controls received (+) DMPA [n=2] or (-) DMPA, [n=3] only. The reproductive tracts were removed one to three months after treatment for examination.

Results: No fallopian tubal occlusion was observed in negative controls (+/- DMPA). Histologic complete tubal occlusion was observed in 3/8 of females treated with 1% PF and 6/7 treated with 5% PF. Histologic evaluation suggested that 1% PF is associated with prolonged chronic inflammation (> 2-3 months), while 5% treatment eliminates the epithelial lining, at least focally, and resolves into complete occlusion within 1-2 months. This pattern of complete occlusion was seen in all 4 females that received 5 % PF (+DMPA), and in 2/3 that received 5% PF (-DMPA).

Conclusion: In a baboon model of transcervical permanent contraception, a single treatment with 5% PF resulted in complete tubal occlusion more reliably (85%) than 1% PF (38%). Co-treatment with DMPA may improve treatment results with 5% PF but requires additional study.

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73 Implications: A finding that a single transcervical treatment with 5% polidocanol foam can
74 occlude the fallopian tubes of baboon supports further study of this approach as a novel
75 strategy for permanent contraception for women.

1. Introduction

The development of a safe, low-cost nonsurgical method of permanent contraception (PC) has been a goal of family planning investigators for more than 40 years [1]. A number of agents have been evaluated for their potential to occlude fallopian tubes after transcervical administration directly into the uterine cavity. The greatest challenge for these approaches has been to achieve high rates of bilateral tubal occlusion following a single treatment [2]. The most extensive experience has been with quinacine sterilization (QS), a technique originally developed in the 1970s, with multiple human studies including large clinical trials [3-6]. However, even with two applications of QS, the failure rate was 2-4 times greater than surgical tubal ligation by 10 years [7, 8].

Our group has been investigating transcervical administration of polidocanol as a strategy for PC. Polidocanol (hydroxy-polyethoxy-dodecane) is a synthetic long-chain fatty alcohol originally developed and marketed as an injectable local anesthetic [9]. In 2010, 0.5% and 1% polidocanol solutions received FDA approval as a sclerosing therapy for the treatment of uncomplicated “spider” and “reticular” varicose veins up to 3mm in diameter (Asclera™, BioForm Medical, Inc). The efficacy of the sclerotherapy can be improved when polidocanol is administered as a foam [10-12]. The scientific basis of this approach is that the foam state provides a greater surface concentration of polidocanol at the point of contact with the endothelium without increasing the total dose. Recently, in 2013, the FDA approved 1% PF (Varithena, Biocompatibles, Inc.) for venous sclerotherapy.

We have previously shown that transcervical administration of 5% polidocanol foam (PF) to macaques produced bilateral tubal occlusion, but multiple treatments were required to assure full tubal blockade [13]. While rhesus macaques are excellent nonhuman primate (NHP) models for human reproductive physiology, transcervical approaches are compromised by the

torturous nature of the macaque cervix [14]. In contrast, the baboon displays a straight cervical canal, similar to women, that facilitates transcervical approaches [14]. The anatomy and physiology of the baboon fallopian tube is also similar to women [15], [16]. Most significantly for studies of permanent contraception, the nonhuman primate fallopian tube passes through a thick muscular area of the uterine wall (the intramural portion) as is true in women. This anatomy is not seen in rodents and domestic animals (e.g. sheep, dogs, cattle). [15, 16]

In this study we evaluated two concentrations of PF in the baboon to determine if a single treatment approach was feasible. We combined the treatment with depomedroxyprogesterone acetate (DMPA) in some animals to control timing of the evaluation in the menstrual cycle.

2. Methods

2.1 Animal care

All study procedures were approved by the Southwest National Primate Research Center (SNPRC) Institutional Animal Care and Use Committee. Twenty adult female baboons (*Papio anubis*, *Papio hamadryus*) between the ages of 7-19 years were used in this study. Animal husbandry provided by SNPRC is in accord with the National Institutes of Health (NIH) Guidelines for Care and Use of Laboratory Animals [17]. Menstrual cyclicity was monitored by evaluation of sex skin characteristics and confirmed by measurement of serum estradiol (E₂) and progesterone (P). Serum samples were stored at -80C and shipped to the Endocrine Services Core Laboratory, Oregon National Primate Research Center (ONPRC) where hormone assays were performed using an electrochemoluminescent Roche Elecsys 2010 analyzer (F. Hoffmann-La Roche Ltd, Basel Switzerland).

In an effort to standardize our evaluation of animals (e.g. evaluate all animals with a thin inactive endometrium), we treated some females with a single dose of depo medroxyprogesterone

acetate (DMPA, 3.5 mg/kg, IM; Pharma & Upjohn (Pfizer), New York, NY) prior to the initial examination. Other females received the same dose of DMPA immediately following the initial PF treatment.

2.2 Hysterosalpingogram procedure

Most of the animals in this study underwent a hysterosalpingogram (HSG) procedure immediately prior to PF infusion and then a repeat HSG evaluation 1-2 months after the initial procedure. If the HSG evaluation showed unilateral or bilateral tubal patency, PF treatment was repeated. To conduct the HSG the animals underwent sedation with ketamine (10 mg/kg IM, Putney, Dublin, OH) and then anesthesia was induced with isoflurane (WI, Boise, ID) inhalation. While in dorsal lithotomy, the vulva and vagina were sterilely prepped and the cervix was visualized with a speculum and grasped with forceps. Under transabdominal ultrasound guidance, the cervix was dilated until 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 balloon was inflated with 0.4 to 1.0 mL of saline, and correct position was confirmed by observing saline distention of the cavity by ultrasound. A series of digital radiographs were obtained to evaluate tubal patency. The images were acquired and instantly reviewed before and following the infusion of small aliquots (1-3mL) of radiopaque contrast (Isovue®, iopamidol injection, Bracco Diagnostics, Monroe Township, NJ) through the catheter until either bilateral tubal patency, substantial vascular uptake without tubal patency, 20 mL of contrast infusion, or balloon expulsion through the cervix was observed.

2.3 Polidocanol foam treatment

Polidocanol 1% and 5% solutions were prepared by mixing polidocanol stock (Sigma P9641) with sterile physiologic buffered saline. Foam was created using the two syringe technique originally described by Cavezzi and Tessari [18]. A total of 2mL of polidocanol 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 to the HSG catheter, and foam was instilled into the uterine cavity under ultrasound guidance. Typically, foam was infused until uterine pressure led to backflow of foam through the cervix or until 16 mL of solution (80mL foam) was administered. After the procedure, animals received routine post-operative care, and were returned to standard husbandry within 24 hours. Control females did not receive foam infusion.

2.4 Experimental groups

The control group consisted of 5 females that did not receive intrauterine PF prior to necropsy. Two controls received DMPA and three did not receive DMPA. Eight females were treated with 1% PF. Five animals in this group received DMPA prior to (23 days, n=2), or immediately following (n=3) the HSG procedure. Three other 1% PF cases did not receive DMPA. The 5% PF group (n=7) included four animals that received DMPA prior to (6- 7 days, n=3), or immediately following (n=1) the HSG procedure; and three females that did not receive DMPA. Two of these animals did not undergo a repeat HSG evaluation, but all were evaluated for patency at the time of necropsy.

2.5 Gross and histologic examination of fallopian tube

A gross post mortem evaluation was performed for all animals by veterinary pathologists at the SNPRC Pathology Services Unit according to their standard necropsy protocol. The uterus and cervix, upper vagina, bilateral tubes and ovaries were extirpated as a single specimen, inspected for gross anatomical abnormalities, and placed in a physiologic saline solution (Hank's Balanced Salt Solution; Sigma). A HSG catheter was then placed transcervically and indigo carmine instilled (*ex vivo*) to assess tubal patency. Further dissection was performed under 2.5 x magnification using a dissecting microscope to free the fallopian tube from the

mesosalpinx, broad ligament and ovary. The uterus was bisected and one of the cornual regions was paraformaldehyde-fixed and paraffin-embedded for histologic sections. The other was flash frozen for future studies. Multiple leveled sections were stained for hematoxylin and eosin to evaluate fallopian tube histology [17]. Representative sections were further evaluated by a gynecologic pathologist while blinded to treatment group. Sections were scored by the pathologist for 1) tubal patency (an obliterated lumen in at least a focal 10x objective field warranted a diagnosis of complete occlusion); 2) inflammation (acute, chronic, or eosinophilic); 3) epithelial damage or reactive metaplasia; 4) damage to the tubal wall associated with foamy histiocytic reaction. Results were reported as dichotomized yes or no and summarized in Table 1. In cases of partial occlusion, the lumen lining epithelium was highlighted by immunostaining for cytokeratin, using routine methods in our laboratory [19].

2.6 Data analysis

The primary endpoints were the frequency of functional blockade assessed by HSG (or dye infusion) and the histologic assessment of tubal occlusion at necropsy. The study was designed as a descriptive project to demonstrate proof of concept that a single administration of polidocanol foam can occlude the baboon oviduct. Three animals per treatment was considered the minimum needed to establish the technique, and to exclude biologic variability as a factor in the histological outcome. Sample size for this pilot study is insufficient for statistical analysis.

3. Results

3.1 Clinical assessment of tubal patency

Table 1 compares the outcome of initial and final clinical evaluation of tubal patency with the final histological assessment. Out of the 20 animals evaluated, 18 underwent a baseline assessment of tubal patency by HSG. Bilateral patency was seen in 13/18 (72%), unilateral patency was observed in 3/18 (17%), and bilateral occlusion was present in 2/18 (11%).

Control females did not undergo additional treatment, and received only one assessment of tubal patency prior to necropsy. Uterine size and compliance were subjectively reduced in baboons that had received DMPA more than 8 days prior to evaluation. For this reason, we were unable to confirm tubal patency in several animals that were sent to necropsy as untreated controls and subsequently found to have histologically open tubes (see below). Except for this DMPA effect, the histologic evaluation of patency (Table 1) correlated with the clinical assessment. Typically, the *ex vivo* assessment of patency confirmed the findings at HSG.

All but two of 15 animals treated with PF underwent at least one follow-up HSG assessment 34-146 days after the initial treatment (Table 1). Bilateral or unilateral patency was observed at the initial follow-up HSG in 3 animals, and each received a second treatment with the same concentration of PF followed by a third HSG. Bilateral occlusion was noted in the other 10 females; six underwent necropsy immediately following the second HSG exam and four underwent a third HSG. All but one female was noted to have bilateral occlusion on the final assessment by HSG. Two 5% PF-treated females did not undergo a final HSG exam; one had bilateral occlusion, and one unilateral patency on the *ex vivo* dye test.

3.2 Histologic assessment of tubal patency

All five control females showed normal tubal epithelium and patent fallopian tubes (Fig. 1). Polidocanol foam treatment (1% and 5%) resulted in histologic changes confined to the intramural portion of the fallopian tube. A variety of changes of increasing severity were seen ranging from subtle thickening of the basement membrane with otherwise normal epithelium (Fig 2a) to complete obliteration of the lumen with scarring and no identifiable epithelium (Fig 2b, 2c). A pattern of occlusion with marked edema and chronic inflammation was seen in some cases associated with foamy histiocytosis (Fig 2d). In some of these cases, focal areas of incomplete luminal occlusion were associated with nests of cells that immunostained positive for

cytokeratin, an epithelial cell marker (Fig 2e). These foci may represent either incompletely occluded lumens with reactive squamous metaplasia (Fig 2f), or attempts at recanalization of the chronically inflamed tissue. Since these samples represent “snap shots” in time, this point is unresolved. Importantly, when complete occlusion of any type developed, it typically occurred in a narrow region of the middle portion of the intramural zone, and was bounded by normal epithelium and lumen at the proximal (uterine) and distal (isthmic) ends.

3.3 Effect of polidocanol dose and DMPA

The treatment effects on the fallopian tube epithelium, time course of inflammatory pattern, and ability to completely occlude the lumen were affected by PF dose (Table 1). Treatment with 1% PF resulted in bilateral or unilateral occlusion by clinical assessment in 5/8 females, but histology confirmed this occlusion in only 3/8 (38%). Two of these three showed the pattern of occlusion with marked edema and chronic inflammation associated with foamy histiocytosis (Fig 2c). Notably, two of the 1% PF animals were treated twice. One showed bilateral patency and normal histology and the other had bilateral occlusion with marked edema and chronic inflammation associated with foamy histiocytosis.

In contrast, 6/7 (86%) 5% PF-treated animals showed bilateral occlusion by final HSG and dye test. The one animal that showed unilateral patency on HSG and dye test had histologic evidence of a normal tube on one side and a complete occlusion with scar on the other. One female treated twice with 5% PF showed an unusual pattern of occlusion associated with marked edema, prominent inflammation and an eosinophilic infiltrate surrounding compacted epithelial cells (Fig 2e). The remaining five 5% PF-treated females were treated only once and showed complete occlusion with scar, suggesting single dose administration with this concentration may be sufficient to achieve permanent contraception.

Treatment with DMPA alone did not alter the histology of control females (Fig 1). Moreover, co-treatment of DMPA plus 1% PF did not increase the rate of histological or clinical occlusion. However, all 4 females that received DMPA plus a single treatment with 5 % PF developed clinical bilateral tubal blockade detected by HSG and histologic evidence of complete tubal occlusion with scar (Table 1). Although tubal occlusion also developed in the three 5% PF treated females that did not receive DMPA, only one developed complete occlusion with scar. The occlusion pattern associated with edema, chronic inflammation and foamy histiocytes was noted in one female after two treatments, and one other female developed only unilateral occlusion. Taken together, these data suggest 5% plus DMPA may be more effective.

4. Discussion

Earlier studies in the rhesus macaque demonstrated 5% PF can block the intramural portion of the fallopian tube after multiple treatments [13]. Our goal was to determine whether a single PF treatment could induce permanent tubal blockade. In this work we utilized the baboon as an animal model because this species has a cervix with a straight path similar to women that provides easier access to routine dilation and intrauterine procedures. We conclude that the baboon is a superior animal model for these studies. However, cycle phase and treatment with DMPA were found to affect clinical evaluation of tubal patency by HSG and *ex-vivo* dye infusion. Histological assessment of tubal patency appeared to be more specific than HSG.

Our results demonstrate that most baboons treated with a single 5% PF treatment will develop dense tubal occlusion, and that this dose is superior to 1% PF. One possible explanation for this enhanced activity may be the slower dissolution of bubbles with the higher concentration foam. This would allow the active agent to remain in contact with the tubal epithelium for a longer time possibly leading to greater or deeper damage and more rapid time course with relatively minimal inflammation after 1-2 months and complete tubal occlusion.

284

285 While the assessment of DMPA is limited in this study by small sample size, results with the 5%
286 PF plus DMPA group were notable in that complete occlusion with scarring occurred after only
287 one treatment in all animals, the only treatment group with this finding. These results support
288 further study into the effects of DMPA combined with 5% PF. No independent effect on tubal
289 occlusion is seen with DMPA, as tubal occlusion did not develop in control females that did not
290 receive PF. And, DMPA treatment did not improve results with 1% PF, which suggests the
291 development of permanent tubal occlusion requires a sufficient degree of tubal injury more likely
292 with 5% PF.

293

294 If proven in a larger series of animals, the single dose approach of 5% PF plus DMPA may have
295 several potential benefits. First, it would provide a direct clinical benefit by providing simple,
296 highly effective low cost short-term contraceptive protection while permanent contraception is
297 achieved through tubal blockade. Second, DMPA would control the hormonal *milieu* during the
298 occlusion process. Medroxyprogesterone acetate is a complex progestin that may have
299 multiple actions [20]. It can act directly through binding to progesterone, androgen and
300 glucocorticoid receptors in target cells, or indirectly by reducing ovarian estradiol production
301 [20].

302

303 The biology of tubal occlusion is poorly understood. Agent-induced damage to the tubal
304 epithelium is normally followed by re-epithelialization and repair [13]. The narrow intramural
305 portion of the tube appears to be the region most vulnerable to damage from sclerosing agents.
306 If damage extends to the subepithelial muscle layer, collagen replacement appears to prevent
307 regrowth of epithelium resulting in occlusion [14]. Conditions that favor collagen production and
308 inhibit epithelial regeneration may improve the efficacy of transcervical PC procedures. Tubal
309 epithelium expresses estrogen receptors and estrogen may have a role in proliferation of the

tubal epithelium [15] and repair and recanalization. Progesterone withdrawal at the end of the cycle may also play an important role [16].

Major limitations of this study include a small sample size insufficient for statistical analysis, non-uniformity of time points for evaluation, and (in most cases) histologic assessment of only one fallopian tube. A strength is the use of the highly relevant baboon translational model with cervical anatomy suitable for evaluating transcervical procedures, and fallopian tube anatomy that includes the critical intramural segment, a unique feature of primates. While our results demonstrate the potential of a single dose nonsurgical approach for permanent contraception, our impressions are based on gross and histologic evaluation of tubal occlusion as the outcome. A prospective contraceptive study with pregnancy as the outcome metric will more directly test our conclusions.

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Figure and Table legends

Figure 1. Representative longitudinal sections from the intramural portion of the fallopian tube from control females that did not receive polidocanol foam. A single layer of normal epithelium is present and the lumen is patent. Detail shown in insert. a) Animal treated with depomedroxyprogesterone acetate (DMPA) 16 days prior to necropsy. b) No DMPA treatment.

Figure 2. Representative longitudinal (a,b,d,f) and cross (c,e) sections from females treated with polidocanol foam (PF). Detail shown in inserts. a) Early changes of basement membrane thickening associated with normal epithelium and tubal patency were commonly observed in PF treated animals without occlusion. b) Complete occlusion associated with loss of tubal epithelium and scaring characteristic of treatment with 5% polidocanol foam and DMPA. c) Cross section of complete occlusion with chronic inflammation. d) Occlusion associated with edema, chronic inflammation and foamy histiocytosis characteristic of 1% PF treatment. e) Positive immunohistochemistry staining for cytokeratin demonstrates the presence of epithelial cells (arrow) in an area of foamy histiocytosis. f) Occlusion associated with edema, chronic inflammation and squamous metaplasia.

Table 1. Clinical and histologic assessment of tubal patency. Female baboons underwent clinical evaluation of tubal patency by hysterosalpingogram (HSG) or by an intrauterine infusion of dye following necropsy (indicated by an asterisk [*]). Treatment with polidocanol foam occurred immediately following the baseline clinical assessment, and was repeated in some animals (indicated by the grey cells). The timing (in days) of administration of depomedroxyprogesterone acetate (DMPA) and necropsy is relative to the baseline assessment. Control (untreated) females underwent necropsy of the day of the initial evaluation and did not undergo a subsequent evaluation. Some of the PF treated females underwent necropsy after the second evaluation. The histologic assessment of patency was OPEN if no area of obstruction or epithelial damage was observed, INFLAM if occlusion associated with marked edema, chronic inflammation and foamy histiocytosis was seen, and COMPLETE if complete occlusion when scaring and no identifiable tubal epithelium was observed.

Table 1.		Clinical evaluation of tubal patency			necropsy (days)	
Animal ID	DMPA (days)	Baseline	Second assessment	Third assessment		Histology
Control						
18XX4	none	bilateral patency*			0	OPEN
14XX0	none	Not assessed			0	OPEN
28XX2	none	bilateral occlusion			0	OPEN
27XX4	yes (-15)	unilateral patency			0	OPEN
15XX2	yes (-16)	bilateral occlusion			0	OPEN
1% Polidocanol Foam						
26XX3	none	bilateral patency	bilateral patency	bilateral patency	97	COMPLETE
27XX7	none	bilateral patency	unilateral patency	bilateral occlusion	97	INFLAM
27XX4	none	bilateral patency	bilateral occlusion	bilateral occlusion	98	INFLAM
27XX3	yes (0)	bilateral patency	bilateral occlusion		146	OPEN
13XX0	yes (0)	bilateral patency	bilateral occlusion		146	OPEN
14XX6	yes (0)	bilateral patency	bilateral occlusion		146	OPEN
27XX7	yes (-23)	unilateral patency	bilateral occlusion	bilateral patency	82	OPEN
27XX1	yes (-23)	unilateral patency	bilateral occlusion	unilateral patency	82	COMPLETE
5% Polidocanol Foam						
11XX7	none	bilateral patency	bilateral patency	bilateral occlusion	145	INFLAM
16XX7	none	bilateral patency	unilateral patency*		47	unilateral COMPLETE
19XX7	none	bilateral patency	bilateral occlusion*		47	COMPLETE
27XX1	yes (0)	bilateral patency	bilateral occlusion		37	COMPLETE
19XX2	yes (-6)	bilateral patency	bilateral occlusion		37	COMPLETE
18XX8	yes (-7)	bilateral patency	bilateral occlusion		37	COMPLETE
27XX2	yes (-7)	bilateral patency	bilateral occlusion	bilateral occlusion	86	dense occlusion

Figure 1

Figure 2