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The Effects of Transcervical Monofilament Insertion on the Microbial Status of the Uterus in Guinea-pigs

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Abstract—The monofilament marker tail attached to intra-uterine contraceptive devices (IUCDs) has been implicated in the development of pelvic infection because it acts as a substrate for bacterial adhesion, and facilitates the transmission of vaginal bacteria into the uterus. A guinea-pig model was used to investigate the role of transcervical monofilaments in the transmission of vaginal bacteria into the uterus. By 21 days the degree of uterine contamination was significantly lower than after 24 h ($P < 0.05$), but was still significantly greater than control values ($P < 0.01$). Coating the monofilaments with poly (2-hydroxyethyl-methacrylate) (polyHEMA) had no effect on uterine bacterial counts at 24 h. After 21 days, however, contamination within the uteri fitted with polyHEMA-coated monofilaments had significantly increased from levels observed at 24 h ($P < 0.01$). These counts were also significantly greater than those recorded for the uncoated and control groups at the same time interval. In animals fitted with monofilaments, in which chlorhexidine had been incorporated into the polyHEMA coating, the level of uterine contamination after 24 h was significantly lower than that observed with uncoated threads at the same time interval ($P < 0.02$). After 21 days there was no significant difference between uterine bacterial counts from the chlorhexidine group and control animals. It is concluded that the use of such drug-loaded monofilaments offers the potential to minimize infections associated with the transcervical insertion of IUCDs.

The normal microflora of the human vagina and cervix is extremely complex comprising interacting and competing microorganisms. These bacteria are subject to an array of hormonal and chemical interactions which result in short-term cyclical changes and long-term, age-related changes. The microbial status of the uterus has proved more difficult to assess due to problems associated with the recovery of samples from that area. For example, it is likely that samples taken by a transcervical route will be affected by contamination from the cervix. The most reliable information has therefore been gained from hysterectomy patients, where the problem of cervical contamination is eliminated. Sparks et al (1977) studied fifty hysterectomy specimens by a multiple biopsy technique, and found that in all cases the uterine cavity was sterile. The sterility of the uterus is maintained by the cervix, the upper half of which (the internal os) is closed except during ovulation and menstruation. In addition, there is a local secretory immune system in the cervix producing secretory IgA which, in the presence of complement and lysozyme, destroys bacteria, blocks bacterial adhesion to mucosal cells and promotes agglutination and phagocytosis. Cervical mucus viscoelasticity also provides a physiochemical barrier to the entry of both vaginal and cervical microorganisms into the uterus, although at ovulation there is a decrease in viscoelasticity corresponding to an increase in sperm and microbial penetration (Elstein 1978; Weström 1987).

All methods of contraception are associated with the risk of side-effects, complications and the failure to prevent pregnancy. Significant risks related to the use of intra-uterine contraceptive devices (IUCDs) include pelvic inflammatory disease, tubal infertility, ectopic pregnancy, uterine perforation, bleeding and pain. Pelvic inflammatory disease is defined as the acute syndrome associated with the ascending spread of microorganisms from the lower to the upper female genital tract (Weström 1987). The potential association between use of IUCDs and the development of pelvic inflammatory disease has been the subject of a large number of studies. The most objective of these studies indicate an increased risk among IUCD users of 1·5–2·6 compared with women using no method of contraception (Grimes 1987). All IUCDs currently marketed in the UK possess a monofilament marker tail, to ease their location and removal, while also providing a means to confirm that the device is still in place. It has been suggested that these monofilaments interfere with the protective mechanisms of the female genital tract by facilitating the ascent of bacteria into the uterine cavity, and thus contribute to the aetiology of pelvic inflammatory disease (Sparks et al 1981; Skangalis et al 1982). Support for this hypothesis, however, has come mainly from in-vitro studies which have investigated the migration of bacteria along IUCD monofilaments coated with cervical mucus (Purrier et al 1979), and through gel systems in the presence or absence of various polymer monofilament threads (Wilkins et al 1990). Problems arise in extrapolating results from such studies to in-vivo situations where conditions are likely to be different; for example, the surfaces encountered by the bacteria will be subject to bioconversion due to the adsorption of biological macromolecules from the surrounding environment.

In the studies conducted here, a guinea-pig model, as described by Gard et al (1993), was used in an attempt to investigate the role of transcervical monofilaments in the transmission of microorganisms from the microbial environment of the vagina into the normally sterile uterus. This in-vivo model was also used to study the effects of coating the monofilaments with poly (2-hydroxyethyl methacrylate) (polyHEMA) and incorporating chlorhexidine into this

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coating on the survival of those bacteria carried into the uterus.

Materials and Methods

Preparation of monofilaments

Nylon monofilaments were washed in a 1% (v/v) Decon solution (BDH Chemicals Ltd, Poole, Dorset, UK) for 2 h. They were then rinsed thoroughly in distilled water, and finally soaked in distilled water (frequently changed) for at least 24 h. Before insertion, monofilaments were treated by placing them under a bactericidal UV light unit (Hanovia Lamps, Slough, Berks, UK) at a distance of 20 cm for 1 h (with occasional rotation). PolyHEMA-coated monofilaments were prepared by dip-coating in a 5% (w/v) polyHEMA (Aldrich Chemical Company, Gillingham, Dorset, UK) solution in 95% (w/v) ethanol, followed by air-drying at room temperature (21°C) for 1 h. The coated monofilaments were then treated by exposure to UV radiation, as before, and equilibrated in sterile water for at least 24 h before use. Chlorhexidine-impregnated monofilaments were prepared by swelling polyHEMA-coated threads in a 20% (w/v) chlorhexidine digluconate solution for 24 h. Before insertion, these drug-loaded monofilaments were treated by exposure to UV radiation as before.

Insertion of monofilaments into uteri

Virgin female Dunkin-Hartley guinea-pigs, 300–500 g, housed on wire-bottomed cages, were used for experimentation. Neuroleptic analgesia was induced by intraperitoneal administration (2·5 mg kg^{-1}) of diazepam (Roche Products Ltd, Welwyn Garden City, Herts, UK), followed by intramuscular administration (0·5 mL kg^{-1}) of Hypnorm (Janssen Pharmaceutical Ltd, Oxford, UK), a mixture of fentanyl citrate (0·315 mg mL^{-1}) and fluanisone (10 mg mL^{-1}). Following vaginal lavage with 0·05% v/v chlorhexidine gluconate solution, the cervix was visualized using a modified Kilian nasal speculum with a fibre-optic light source. The reproductive system of a female guinea-pig consists of two uterine horns that are connected to the vagina by a single cervix. A device constructed with a sterile glass capillary tube (i.d., 1·4 mm) and sterile nylon tubing (i.d., 1 mm) was used to house the monofilament (Fig. 1), and this was introduced into one of the uterine horns via the cervix. Following laparotomy one horn of the bicornate uterus was exposed and the monofilament anchored to the wall of the uterus by a double ligature using silk sutures (Ethicon Ltd, Edinburgh, UK). Once the monofilament was in position, the capillary tube and the nylon tubing were withdrawn from the uterus and discarded. The abdominal lesion was then repaired using silk and nylon sutures, a microporous dressing was applied and the animals were left to recover in a warm environment, for approximately 24 h.

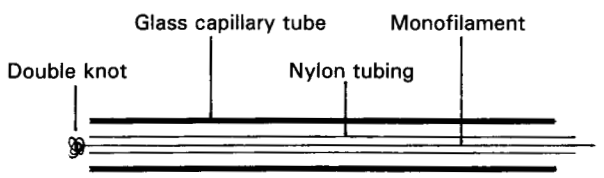

FIG. 1. Diagram of device used for the insertion of the monofilament into the uterus.

Assessment of microbial status of uteri

At selected time intervals, the animals were killed and their uteri examined for the presence or absence of microorganisms. The bicornate uterus was dissected out under aseptic conditions. The horn which held the monofilament was washed repeatedly with 2 mL quarter-strength Ringer solution. Following appropriate dilution of the resultant cell suspension, samples (0·2 mL) of each dilution were plated onto overdried nutrient agar plates using sterile glass spreaders. The plates were then incubated at 37°C for 24–48 h and the number of colony-forming units per mL (cfu mL^{-1}) of original wash suspension determined. The other horn was incubated whole in nutrient broth at 37°C for 24 h when it was tested for bacterial contamination by the presence or absence of turbidity. (In those experiments involving chlorhexidine-impregnated monofilaments 0·1% lecithin and 1% Tween 80 were incorporated into the recovery media.) Control experiments were conducted in the same manner using the uteri of animals in which monofilaments had not been inserted.

All data obtained were statistically analysed using the 2-tailed Mann-Whitney U-test for unpaired non-parametric data (Wardlaw 1987).

Results

The microbial analyses on the uteri removed from guinea-pigs 24 h and 21 days after monofilament insertion are given in Table 1. Results from each individual animal are shown as cfu mL^{-1} of original wash solution for the uterine horn which held the monofilament, and as observations of growth (+) or no growth (−) for the other horn, incubated whole in broth.

Discussion

In the control studies (conducted with animals in which threads had not been inserted) 50% of the uterine washes were sterile and in the remaining washes the degree of contamination was very low. In all cases where the uterine horns were incubated in broth, no bacterial contamination was observed. These results concur with previous studies with guinea-pigs (Malhi et al 1987) and in man (Sparks et al 1977) which showed that under normal circumstances the uterus is sterile.

The insertion of a nylon monofilament into the uterus via a transcervical route resulted in the introduction of microorganisms into the uterus. After 24 h, high bacterial counts were recorded in the majority of the uterine washes, and in all cases the uterine horns incubated in broth were contaminated. It therefore seems likely that bacteria are carried up into the uterus at the time of device insertion, whereupon they can grow and multiply. Bacteria may be taken up into the uterus by the monofilament itself, or on the insertion device which is then removed. However, recovered contamination levels after 21 days were significantly lower ($P < 0·05$) than those obtained after 24 h. This reduction is likely to be due to the action of the host's defence system. Once an IUCD

Table 1. Microbiology of guinea-pig uteri after insertion of monofilaments. Each series of experiments used eight new animals.

	Animals							
	1	2	3	4	5	6	7	8
Control (no insertion)								
Uterine wash (cfu mL^{-1})	0	0	0	0	5	5	10	15
Uterus in broth (±)	−	−	−	−	−	−	−	−
Nylon monofilaments								
24 h								
Uterine wash (cfu mL^{-1})	10	180	270	1085	$>10^4$	$>10^4$	$>10^4$	$>10^4$
Uterus in broth (±)	+	+	+	+	+	+	+	+
21 days								
Uterine wash (cfu mL^{-1})	35	38	45	80	98	130	257	265
Uterus in broth (±)	−	+	−	+	+	+	+	+
PolyHEMA-coated nylon monofilaments								
24 h								
Uterine wash (cfu mL^{-1})	10	15	30	100	120	250	2010	$>10^4$
Uterus in broth (±)	−	−	+	+	+	+	+	+
21 days								
Uterine wash (cfu mL^{-1})	1750	1850	4600	$>10^4$	$>10^4$	$>10^4$	$>10^4$	$>10^4$
Uterus in broth (±)	+	−	+	+	+	+	+	+
Chlorhexidine-impregnated monofilaments								
24 h								
Uterine wash (cfu mL^{-1})	0	10	20	55	75	95	175	180
Uterus in broth (±)	−	−	−	+	+	−	−	−
21 days								
Uterine wash (cfu mL^{-1})	0	0	5	10	15	30	30	45
Uterus in broth (±)	−	−	−	−	−	−	−	−

has been placed into the uterus it induces an inflammatory response (Spence 1984) and, indeed, this has often been related to the antifertility effects of such devices (Hudson & Crugnola 1987; Johannisson 1987). Such a foreign body reaction has been shown to result in elevated levels of intrauterine leucocytes (El Sahwi & Moyer 1970) and macrophages (Sagiroglu & Sagiroglu 1970), and these findings are supported by electron microscopy studies performed on various inert IUCDs which were removed from uteri immediately after hysterectomy (Sheppard & Bonnar 1980). The cellular material on the surface of these devices consisted mainly of macrophages, with some polymorphonuclear leucocytes, erythrocytes, platelets and fibrin fibres. The presence of these inflammatory cells in the uterine environment is likely to lead to the elimination of bacteria introduced at the time of monofilament insertion, and is therefore probably responsible for the reduction in bacterial levels seen in uterine washes after 21 days. It is also possible that the damage caused to the uterus wall while anchoring the monofilament with sutures, may allow the passage of inflammatory cells into the uterine cavity and thus further facilitate bacterial removal.

Although uterine bacterial levels after 21 days are significantly lower than those observed after 24 h, they are still significantly greater than control values, where no monofilament was present ($P < 0{\cdot}01$). These results are in contrast to a similar study with guinea-pigs when Gard et al (1993) observed no significant reduction in contamination levels between 24 h and 21 days, and suggested that bacteria may be protected from host defences by a biofilm which had adhered to the surface of the monofilament. With respect to the present study, it is conceivable that on first insertion, bacteria are carried up into the uterus somewhat loosely attached to a film of mucus, and after 24 h these are relatively easily removed from the monofilament and bacterial counts in the uterus are high. After 21 days, however, the cells may have become firmly embedded in the biofilm and are hence much more difficult to dislodge by the washing procedures employed, therefore giving an artificially low microbial count. The possibility that the monofilament marker tail, attached to IUCDs, may facilitate the transmission of bacteria from the vagina to the uterus is well documented in the literature (Spence 1984; Weström 1987). In-vitro studies, involving a model simulating the female genital tract, have shown that bacteria adhere to and can migrate along polymer monofilament threads (Wilkins et al 1989, 1990). In-vivo studies, with guinea-pigs, examined the effect of cutting inserted monofilaments level with the neck of the cervix, and found that the presence or absence of a thread in the vagina did not influence the degree of uterine contamination (Gard et al 1993). This evidence does not exclude completely the possibility that bacteria present within the cervix may progress along the monofilament surface and into the uterus. However, it has already been suggested that the microbial contamination present after 21 days could be due to bacteria introduced during insertion and possibly afforded protection by material coated on the thread surface.

Previous in-vitro studies have demonstrated that coating nylon monofilaments with polyHEMA significantly reduced bacterial adhesion to the thread surface (Bilbruck et al 1993). It was, therefore, hoped that by inserting polyHEMA-coated monofilaments, uterine contamination would be less than that observed with untreated threads. However, after 24 h, bacterial counts in the uteri of the polyHEMA group were not significantly different from those obtained with uncoated nylon monofilaments at the same time interval. Also, after 21

days, uterine contamination, within the polyHEMA-coated monofilament group, showed a significant increase from levels after 24 h ($P < 0.01$), and was significantly greater than the colonization observed in the uteri of the uncoated group ($P < 0.01$). A possible explanation for these high bacterial counts may be derived from possible disruptions to the polyHEMA coating that could feasibly allow microorganisms beneath the polyHEMA coat, where they might ascend the monofilament, by capillary action. Alternatively, the inflammatory response induced by the presence of the monofilament may be reduced when it is coated with polyHEMA. For example, Singh & Melrose (1971) described how polyHEMA-coated sutures almost eliminated the strong foreign-body reaction found with uncoated sutures, while another study has reported that polyHEMA-coated IUCDs minimize inflammatory reactions in rabbits (Scott et al 1973).

The level of uterine contamination after 24 h in animals fitted with chlorhexidine impregnated monofilaments, was significantly lower than that observed with uncoated threads at the same time interval ($P < 0.02$). After 21 days there was no significant difference between uterine bacterial counts from the chlorhexidine group and control animals. There was also no contamination observed in any of the uterine horns which were incubated whole in broth. The incorporation of chlorhexidine into monofilaments therefore appears to offer the uterus protection from bacterial colonization. It is likely that, initially, the antibacterial activity of the chlorhexidine will enhance the action of host defences in eliminating uterine contamination. Thereafter, it is possible that the mucospissic effects of chlorhexidine (Chantler et al 1989) retard the ascension of bacteria into the uterus from the cervix and vagina. Support for this hypothesis is provided by Wilkins et al (1989) who showed that increasing the viscosity of carmellose sodium gel, in an in-vitro model, reduced the transmission of bacteria along polymeric monofilaments. However, it should be noted that in some studies, it has been suggested that chlorhexidine could be teratogenic and occasionally sensitivity reactions occur (Senior 1973). The possible risks of using chlorhexidine may, therefore, mitigate against the use of this particular antibacterial agent.

In general, therefore, it seems that in the majority of cases, bacteria entering the uterine cavity as a direct result of IUCD use, whether they be introduced during insertion of the device, or by transmission along the monofilament tail, can be dealt with by host defences. However, in some instances bacteria may multiply beyond the capabilities of the defence system and ultimately lead to pelvic infection. This may occur soon after insertion which implies that it is probably bacteria carried up by the inserted device, but only loosely adhered and therefore easily removed from the substrate surface, that cause infection. There may in some instances, however, be a substantial time interval between insertion and the onset of infection. In these cases bacteria firmly adhered to the surface of the device, and protected from defence mechanisms by a mucus biofilm, could act as a reservoir of infection occasionally breaking away and causing problems. Alternatively, it is possible that delayed colonization is caused by bacteria having ascended from the vagina via the monofilament tail.

In conclusion, it is proposed that the use of polyHEMA-coated monofilaments which allow the incorporation of antibacterial agents onto the thread surface, offer the potential to minimize infections related to the transcervical insertion of IUCDs.

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