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The Effects of Antibiotics and Steroids on the Carriage of Vaginal Bacteria into the Uterus During Insertion of Intra-uterine Monofilaments in the Guinea-pig

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Abstract—The effects of systemic norethisterone acetate and oxytetracycline hydrochloride on the levels of vaginal microorganisms found in the uterus after the insertion of transcervical, intra-uterine monofilaments in the guinea-pig were determined. The results indicated that bacteria were transferred to the uterus from the vagina during the insertion process and, in the presence of an intra-uterine substrate, persisted for up to 6 months. Daily treatment with norethisterone acetate or oxytetracycline hydrochloride whilst the monofilament was in-situ failed to reduce the bacterial numbers in the uterus. Similarly, daily treatment with oxytetracycline hydrochloride for the 5 days before monofilament insertion had no effect on these bacteria.

Women using intra-uterine contraceptive devices (IUCDs) have been assessed as having an increased risk of developing pelvic inflammatory disease (PID) (Senanayake & Kramer 1980; Edelman et al 1982). Reports indicate that the period in which women are most likely to develop PID is that just after insertion of an IUCD (Lee et al 1983). During the first month following insertion the risk of PID is four times greater than in women using no contraception. The relative risk decreases to 1·7 in the second to fourth months after insertion and to unity thereafter (Senanayake & Kramer 1980; Lee et al 1983). Most studies indicate that the risk of developing PID is inversely related to the duration of IUCD use (Lee et al 1983).

It has been suggested that IUCD use may increase the risk of PID in a number of ways. Firstly, the vagina is normally colonized by large numbers of microorganisms and thus vaginal microorganisms may easily be introduced into the uterus during IUCD insertion. The introduced organisms may then give rise to an infection, and it has been shown that commensal organisms are the most common infecting organisms in PID (Westrom & Mardh 1990). Secondly, and related to this, any bacteria that are introduced into the uterus at the time of insertion may be able to withstand the host's immune system by adhesion to the device within a protective biofilm. Evidence in the guinea-pig suggests that transcervical insertion of a monofilament does lead to the introduction of vaginal bacteria into the uterus and that biofilm production occurs but that some bacteria leave the biofilm to become planktonic (Malhi et al 1989). Thirdly, it has been proposed that vaginal bacteria are able to breach the cervical mucus barrier by growth along the IUCD marker tail (Tatum et al 1975). In support of this, in-vitro studies have demonstrated that bacteria are indeed able to progress through a mucus gel to a greater extent in the presence of a nylon or PVDC monofilament than otherwise (Wilkins et al 1989). In the guinea-pig, however, it has been shown that the extent of uterine contamination caused by the insertion of an intra-uterine monofilament is unaffected by whether the monofilament has a transcervical portion or is entirely intra-uterine; this therefore questions the importance of the IUCD marker tail in the aetiology of uterine infections (Gard et al 1993).

Several approaches have been suggested in order to reduce the risk of IUCD-associated PID by decreasing the risk of bacterial transfer from the vagina to the uterus at the time of IUCD insertion. Two of these suggestions are thorough cleaning of the vagina and cervix with an antiseptic, and the prophylactic administration of broad spectrum antibiotics before device insertion (Liskin & Fox 1982). The aim of such actions is to reduce the bacterial population of the vagina and to eliminate rapidly any bacteria that are introduced into the uterus.

Another approach could be to increase the viscoelasticity of cervical mucus by the administration of mucospissic drugs (agents which increase the viscoelasticity of mucus) such as progestogens. As stated previously, it has been suggested that PID may be caused by bacteria breaching the cervical mucus plug following growth along the IUCD marker thread; in-vitro, the bacterial penetration of a gel by growth along a monofilament has been shown to be inversely related to the viscosity of that gel (Wilkins 1986); if such a relationship holds in-vivo, administration of mucospissic drugs may decrease the incidence of bacterial entry into the uterus. Similarly, a mucospissic drug may be able to increase the viscoelasticity of the mucus biofilm surrounding an IUCD, which in turn may decrease the rate at which bacteria leave the biofilm to become planktonic.

The aim of the present study was to use the guinea-pig as a mammalian model to study the insertion of intra-uterine devices as a route of entry for vaginal bacteria into the uterus and to assess the value of the administration of the broad spectrum antibiotic oxytetracycline and the progestogen

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norethisterone in reducing the levels of bacteria isolated from the uterus.

Materials and Methods

Monofilament insertion

Neuroleptanalgesia was induced in virgin female guinea-pigs, 300–500 g, by the administration of diazepam (2·5 mg kg^{-1}, i.p.) and Hypnorm (Janssen Pharmaceutical Limited, UK, 0·5 mL kg^{-1}, i.m.). Following vaginal lavage with 0·05% v/v chlorhexidine gluconate solution, the cervix was visualized using a Kilian nasal speculum, and a sterilized rigid nylon capillary tube housing a nylon monofilament (diam. 0·23 mm) was introduced into the uterus via the cervix. The bicornuate uterus was exposed by laparotomy and the monofilament anchored to the myometrium using a silk suture; the capillary tube was then withdrawn. Monofilaments were trimmed level with the external os of the vagina. The abdominal wound was then repaired with silk and nylon suture. A microporous dressing was applied and the animals were left to recover in a warm environment. In sham-treated animals the monofilament was introduced into the uterus and the sutures passed through the uterine wall, but the monofilament was removed before abdominal repair. At selected time intervals, uteri from eight animals of each group were examined for the presence or absence of microorganisms.

Determination of uterine microflora

The bicornuate uterus was exposed under aseptic conditions. The uterine horn containing the monofilament was cut at the distal region and the intra-uterine portion of the monofilament removed. The lumen of the uterine horn was then washed with 2 mL quarter-strength Ringer's solution and 0·2 mL aliquots of the resultant cell suspension were plated onto nutrient agar and incubated aerobically for 24–48 h at 37°C. The number of colony-forming units (cfu) per mL of original wash suspension was determined. The other horn was removed and incubated whole in nutrient broth at 37°C for 24–48 h when it was assessed for bacterial contamination by the presence or absence of turbidity.

Drug administration

Animals received norethisterone acetate (Sigma Chemical Company Limited, UK) or oxytetracycline hydrochloride (Pfizer Limited, UK) either before or following monofilament insertion. Both drugs were administered intraperitoneally in a saline vehicle at doses of 1·0 and 30·0 mg kg^{-1} day^{-1}, respectively. Five treatment regimes were used: oxytetracycline for 5 days before monofilament insertion; oxytetracycline for 21 days starting on the day of monofilament insertion; oxytetracycline for 5 days before and 21 days after monofilament insertion; norethisterone acetate for 21 days following monofilament insertion; and a combination of norethisterone acetate and oxytetracycline for 21 days following monofilament insertion. The uteri of all animals were examined for the presence of microorganisms 21 days after monofilament insertion.

Results

Microorganisms were found in both uterine horns of all of the sham-treated control animals 1 h, 1 day and 5 days after surgery. These bacteria were of vaginal origin, the microflora of which has been previously characterized (Malhi et al 1987). No uterine contamination was found in those sham-treated control animals investigated 10, 21, 60 days or 6 months after surgery. In contrast, at all time points studied, microorganisms were found in both uterine horns of all control animals with an intra-uterine monofilament in-situ ($P < 0·0001$, vs sham control, Fisher's Exact Test; Table 1).

Pretreatment of the guinea-pigs with oxytetracycline for 5 days before insertion of the monofilaments did not prevent bacteria being carried into the uterus and the numbers found 21 days after surgery were not significantly reduced compared with control values. Similarly, treatment with oxytetracycline for the 21 days during which the monofilament was in-situ or for 5 days before insertion followed by 21 days whilst the monofilament was in-situ had no effect on the number of uteri containing bacteria at the end of the treatment period. Daily administration of norethisterone acetate either alone or in combination with oxytetracycline during the time that the intra-uterine monofilament was in-

Table 2. The effect of norethisterone acetate (1·0 mg kg^{-1} day^{-1}) and oxytetracycline (30·0 mg kg^{-1} day^{-1}) on the extent of uterine contamination in guinea-pigs with transcervical, intra-uterine monofilaments. n = 8 in all cases.

Treatment	Number of contaminated uteri	Mean
Vehicle control	8	190 ± 59
Norethisterone acetate (21 days post-insertion)	8	170 ± 56
Norethisterone acetate + oxytetracycline HCl (21 days post-insertion)	8	190 ± 49
Oxytetracycline HCl (5 days pre-insertion)	8	190 ± 59
Oxytetracycline HCl (21 days post-insertion)	8	222 ± 62
Oxytetracycline HCl (5 days pre- and 21 days post-insertion)	8	199 ± 48

Table 1. The effect of transcervical monofilament insertion on uterine bacteria in the guinea-pig.

Treatment		1 hour	1 day	5 days	10 days	21 days	60 days	6 months
Sham control	Number of contaminated uteri	8	8	8	0	0	0	0
	Mean count (cfu mL^{-1})	229 ± 57	236 ± 86	230 ± 71	0	0	0	0
Transcervical monofilament	Number of contaminated uteri	8	8	8	8*	8*	8*	8*
	Mean count (cfu mL^{-1})	261 ± 43	189 ± 86	246 ± 68	100 ± 86	221 ± 79	193 ± 57	154 ± 54

* $P < 0·0001$, vs sham control. n = 8 in all cases.

situ similarly failed to reduce both the number of uteri containing bacteria and the number of planktonic cells found (Table 2).

Discussion

Guinea-pig uteri are normally devoid of bacterial contamination, although mating has been shown to lead to the introduction of bacteria into the uterus (Malhi et al 1987). These bacteria, however, do not generally lead to infections and they are eliminated rapidly from the uterus. The results for the sham-treated controls indicate that microorganisms were introduced into the uterus during monofilament insertion despite the fact that the materials used were sterile and the vagina had been irrigated extensively with chlorhexidine acetate. However, none of the uteri examined after the 5 day time point contained bacteria indicating that they had been eradicated at some time between 5 and 10 days after introduction. The mechanism of the microbial destruction cannot be elucidated from the results of this study but 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.

The method of estimating the numbers of microorganisms which were present in the uterus took into account only those bacteria which were planktonic. Previous work has shown that large numbers of bacteria are carried up from the vagina into the uterus and are embedded within a mucus biofilm adhered to the substrate. However, these sessile bacteria do not pose any immediate threat and of more concern are those which can break free and become planktonic since these may act as a focus for potential infection. In addition it is these bacteria which can migrate to the contralateral horn.

At all time points tested, bacteria were found associated with all uteri into which monofilaments had been introduced. These results indicate that insertion of the monofilament carries bacteria from the vagina and cervix into the uterus and that these bacteria are able to survive in the presence of a polymeric substrate, probably due to their production of a protective biofilm.

Neither prophylactic nor concurrent administration of the antibiotic oxytetracycline decreased the extent to which vaginal microflora were introduced into the uterus during the insertion of the transcervical monofilament. A previous study has indicated that comparable doses of oxytetracycline in the guinea-pig reduce the number of vaginal bacteria by about 80%, but that the remaining 20% still represent a considerable microbial population (Malhi et al 1988). Thus the inability to totally eradicate vaginal bacteria by administration of oxytetracycline would render the treatment ineffective in preventing the introduction of vaginal bacteria into the uterus at the time of monofilament insertion. It has also been shown that mucus secretions provide a diffusional barrier to oxytetracycline and hence any bacteria embedded within a mucus biofilm are likely to be protected against the antibiotic as well as the host immune system (Costerton et al 1987).

Norethisterone acetate is known to cause an increase in cervical mucus viscoelasticity (Elstein 1974). If microorganisms are able to gain access to the uterus from the vagina by growth along the transcervical thread, then any increase in cervical mucus viscoelasticity may impede their progression. The results indicate that norethisterone acetate, at a dose which has been shown to antagonize the effects of mucolytic agents in the guinea-pig (Malhi et al 1991), had no effect on the uterine contamination. This, together with the finding that the numbers of bacteria isolated from the untreated animals with a monofilament in-situ did not increase over the timescale studied, suggests that the growth of bacteria along a transcervical thread is not a major factor in the continued presence of bacteria within the uterus.

In all of the guinea-pigs treated with norethisterone acetate there was evidence of bacterial contamination of the uterine horn contralateral to that containing the monofilament. This indicates that norethisterone acetate had not prevented bacteria leaving the biofilm associated with the monofilament to become planktonic; however, the method used, presence or absence of turbidity in nutrient broth, does not allow determination of the effect of norethisterone acetate on the numbers of planktonic bacteria. In man, systemic administration of norethisterone acetate, in the form of oral contraceptives, is reported to decrease the incidence of PID amongst non-IUCD users, although no oral contraceptives confer protection if taken for less than 12 months (Panser & Phipps 1991). Over the time span studied, the present results suggest that systemic norethisterone acetate would be unlikely to decrease the incidence or extent of microbial carriage into the uterus following IUCD insertion. This lack of effect may reflect the short duration of treatment or the local drug concentrations achieved as there has been a recent report of decreased incidence of PID amongst users of levonorgestrel-releasing IUCDs (Toivonen et al 1991).

Using the guinea-pig as a mammalian model it seems apparent, therefore, that the presence of vaginal microorganisms within the uterus may be an inevitable consequence of IUCD insertion and that the bacteria introduced may persist for an extended period of time protected within an adhered biofilm. While this may, of itself, not present problems, the ability of some bacteria to break free may enable the biofilm to act as a reservoir of microorganisms which have potential to set up a focus of infection. However, the system described here does not set out to investigate the mechanisms leading to infections and the mere presence of microorganisms is not itself an indicator of infection.

Prior administration of the broad spectrum antibiotic oxytetracycline had no effect on the extent of uterine contamination, possibly due to its inability to reduce sufficiently the vaginal microbial population and because of the diffusional barrier presented by the mucus biofilm. A recent report has recommended that prophylactic antibiotic cover should be given to women upon insertion of IUCDs. However, the data presented here suggest that such a course of action is unlikely to have any significant effect (Centers for Disease Control 1989).

The lack of effect of norethisterone acetate on the extent of microbial contamination of the uterus suggests that the major route of entry for bacteria into the uterus is from the vagina during device insertion. Migration of bacteria from the vagina along the monofilament after insertion does not seem to be of major importance. The microorganisms carried

into the uterus are embedded within a biofilm of mucus and are thus protected from the host defences and can persist for extended periods of time. Whether these vaginal biofilm bacteria can act as a subsequent focus for infection has not been determined and is worthy of further study.

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