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Predisposition to Uterine Microbial Contamination in the Guinea-pig Following Administration of Mucolytic Drugs and Steroid Hormones

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Abstract—Using guinea-pigs as a mammalian model, the effects of bromhexine hydrochloride, ethinyloestradiol, norethisterone acetate and prednisolone acetate on uterine microbial status were determined. Those drugs known to decrease mucus viscoelasticity predisposed to the entry of vaginal bacteria into the uterus, probably due to reduction of the cervical mucus barrier. Norethisterone acetate, which increases cervical mucus viscoelasticity, reduced these effects. The effects produced by the steroid hormones were independent of their immunosuppressive effects. The results also suggest that prednisolone acetate may exert oestrogen-like actions on cervical mucus.

We have previously reported that the mucolytic agents bromhexine hydrochloride and *S*-carboxy-L-methyl cysteine predispose to the transcervical migration of vaginal bacteria into the normally sterile uterus of guinea-pigs (Malhi et al 1987). The mechanism of this effect is unclear although it is probable that these drugs reduce the quantity and viscoelasticity of cervical mucus and thus disrupt a physical barrier which normally prevents the transfer of microorganisms from the microbiologically rich vagina to the uterus. Rheological confirmation of the hypothesis is not possible due to the minute quantities of cervical mucus in guinea-pigs, although these drugs have been shown to produce such effects on mucus secretions from other sites (Richardson & Phipps 1978; Aylward et al 1985; Martin et al 1990).

However, the cervical mucus plug is not the only defence against microbial colonization of the uterus since during the normal sex cycle there is variation in the viscoelasticity of the cervical mucus such that the nadir of viscosity occurs simultaneously with ovulation (Elstein 1978). Sperm penetration is greatest at this time (Elstein 1974; Kerin et al 1976) thus it is likely that the mucus barrier is not completely impenetrable throughout the whole sex cycle, although there is no evidence of microbial colonization of the uterus at the time of ovulation. In addition, it has been shown that vaginal bacteria artifically introduced into the guinea-pig uterus have a survival time of less than 10 days (Malhi et al 1989), illustrating the presence of additional defence mechanisms.

Under normal physiological conditions the quantity, composition and properties of cervical mucus are controlled by sex hormones. Oestrogens cause a decrease in mucus viscoelasticity by inducing the secretion of copious amounts of mucus with a high water content whilst progesterone causes the secretion of a small quantity of highly viscid mucus (Elstein 1974). In common with the other steroid hormones, the sex steroids also induce some degree of immunosuppression due to their effects on leucocyte production (Baxter 1979). Thus oestrogens, by decreasing mucus viscoelasticity and decreasing leucocyte production, compromise two of the defence mechanisms possibly involved in the maintenance of a sterile uterus. Progestogenic hormones, on the other hand, compromise the cellular immune system but potentially increase the effectiveness of the cervical mucus barrier. Prednisolone acetate, a synthetic glucocorticoid hormone, is a potent immunosuppressant, but has no reported effect on the quantity or properties of cervical mucus. By studying the effects of these hormones on the microbial colonization of the uterus it is possible to estimate the relative importance of cervical mucus and the cellular immune system in the maintenance of a sterile uterus under normal physiological conditions.

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Materials and Methods

Experimental animals

Female Dunkin-Hartley guinea-pigs, 300–500 g, were housed in wire-bottom cages in groups of 8 and allowed free access to diet pellets and tap water.

Protocol

Guinea-pigs received mucolytic drugs or steroid hormones intraperitoneally daily for 21 days. Regular total circulating leucocyte counts were made during the course of treatment. At the conclusion of the treatment the uteri were removed and examined for the presence of micro-organisms.

Administration of drugs

Bromhexine hydrochloride (Sigma Chemicals) or steroid hormones were administered daily for 21 days by the intraperitoneal route. The dose of bromhexine hydrochloride was 30 mg kg^{-1} day^{-1}; the steroid hormones used were prednisolone acetate, ethinyloestradiol and norethisterone acetate (Sigma Chemicals) at doses of 2·0, 0·05 and 1·0 mg kg^{-1} day^{-1}, respectively. Further groups of animals received combinations of two of the aforementioned drugs, i.e. bromhexine hydrochloride together with prednisolone acetate, ethinyloestradiol or norethisterone acetate, or ethinyloestradiol together with norethisterone acetate. The control group of animals received 1 mL kg^{-1} 0·1% v/v ethanol vehicle daily for 21 days.

Determination of total leucocyte counts

Blood samples (50 μL) were taken from the marginal ear vein at regular intervals. Following 1 : 20 dilution of the blood in a 2% v/v acetic acid/methylene blue diluent, total leucocyte numbers were determined using a haemocytometer.

Collection and determination of uterine microflora

The guinea-pigs were killed by cervical dislocation and the bicornate uterus rapidly exposed by laparotomy. Both horns of the uterus were removed aseptically and one horn was incubated whole in nutrient broth (Mast Laboratories) at 37°C for 24–48 h; the presence of turbidity in the broth was used to indicate the presence of micro-organisms. The lumen of the other horn of the uterus was repeatedly aspirated with 2 mL quarter-strength Ringer solution. Samples (0·2 mL) of the resultant cell suspension were diluted appropriately and plated onto overdried nutrient agar (Oxoid) plates and incubated aerobically at 37°C for 24–48 h before determination of the number of colony forming units.

Statistical analysis of data

Total leucocyte counts are presented as the group mean plus or minus one standard error of that mean. Treatment groups are compared by two-way analysis of variance.

The incidence of contamination, i.e. the number of uteri exhibiting microbial contamination was compared by use of Fisher's exact test. The extent of uterine contamination, i.e. the mean number of colony forming units per mL of wash suspension, plus or minus one standard error of that mean for each treatment group was compared by use of Student's independent 2-tailed *t*-test.

Results

Total leucocyte count

Bromhexine hydrochloride had no significant effect on the white blood cell counts. When compared with the animals receiving vehicle control, those animals receiving norethisterone acetate and ethinyloestradiol underwent a 20- and 40-fold decrease in white blood cell count, respectively, by the end of the 21 day treatment period. Prednisolone acetate caused a 200-fold decrease in white blood cell count over the same period; these changes were significant at the 0·1% level.

Coadministration of bromhexine hydrochloride with the steroid hormones did not further decrease the white blood cell counts compared with the decrease produced by administration of the steroid hormones alone. Similarly, coadministration of ethinyloestradiol and norethisterone acetate did not produce a greater effect on white blood cell count than was produced by either of these agents alone (Fig. 1).

Uterine microflora

None of the eight guinea-pigs from the vehicle control group showed evidence of uterine contamination. In those groups receiving bromhexine hydrochloride, either alone or in combination with the steroid hormones, or receiving ethinyl-

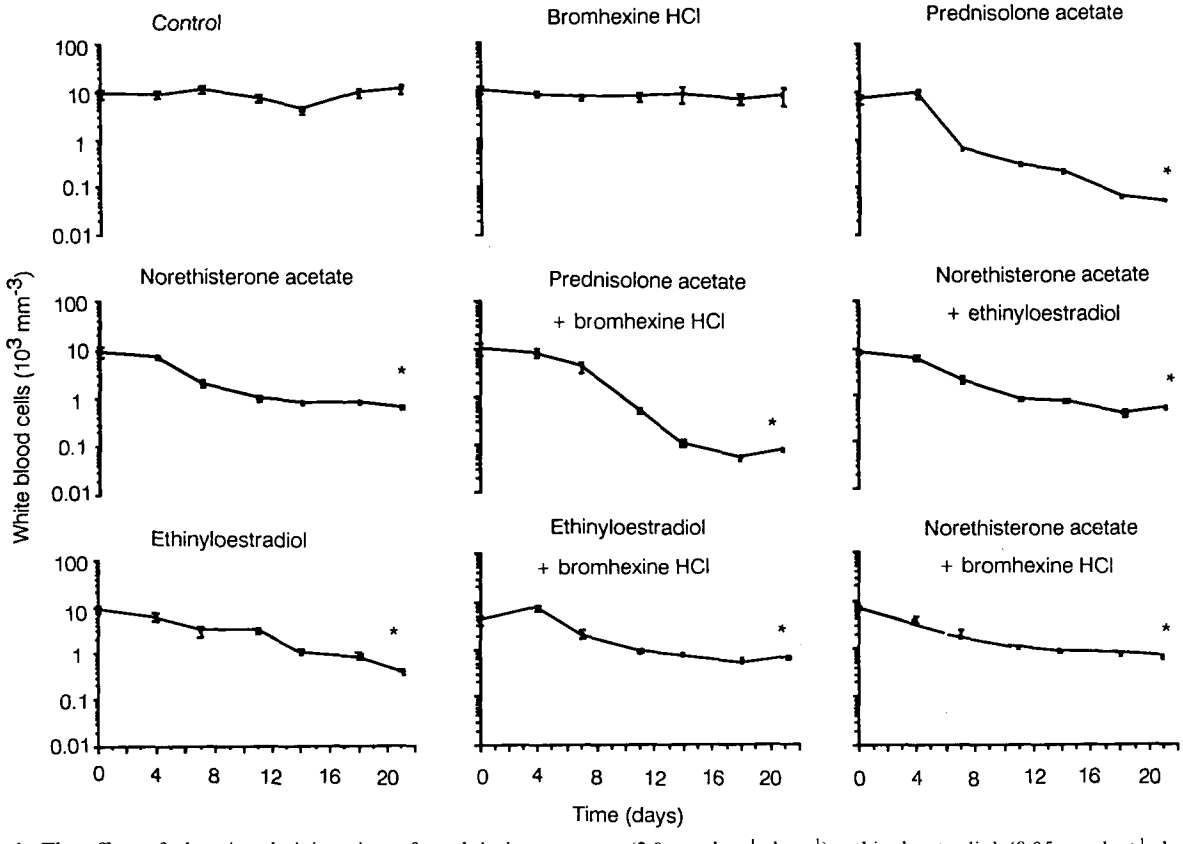

FIG. 1. The effect of chronic administration of prednisolone acetate (2·0 mg kg^{-1} day^{-1}), ethinyloestradiol (0·05 mg kg^{-1} day^{-1}), norethisterone acetate (1·0 mg kg^{-1} day^{-1}) and bromhexine HCl (30 mg kg^{-1} day^{-1}), either alone or in combination, on white blood cell (WBC) count in guinea-pigs. *$P < 0.001$ compared with vehicle control.

Table 1. Incidence of uterine contamination in guinea-pigs receiving prednisolone acetate (2·0 mg kg^{-1}), ethinyloestradiol (0·05 mg kg^{-1}), norethisterone acetate (1 mg kg^{-1}) or bromhexine hydrochloride (30 mg kg^{-1}), either alone or in combination, daily for 21 days.

Treatment	Sample size	Number uteri contaminated
Vehicle control	8	0
Bromhexine HCl	7	7*
Prednisolone acetate	8	3
Ethinyloestradiol	8	8**
Norethisterone acetate	8	0
Ethinyloestradiol + norethisterone acetate	8	1
Bromhexine HCl + prednisolone acetate	7	7**
Bromhexine HCl + ethinyloestradiol	8	8**
Bromhexine HCl + norethisterone acetate	8	4*

* $P<0·05$, ** $P<0·01$ compared with the vehicle control.

oestradiol alone, the number of animals with contaminated uteri was significantly different from vehicle control. Those groups receiving prednisolone acetate or norethisterone acetate, either alone or in combination with ethinyloestradiol, did not show a significantly greater incidence of uterine contamination than the control group (Table 1).

The extent of uterine contamination, as determined by the number of colony forming units per mL of uterine wash solution, was significantly greater in those animals receiving prednisolone acetate and ethinyloestradiol than in those animals receiving vehicle control ($P<0·05$ and $<0·001$, respectively). Norethisterone acetate, either alone or in combination with ethinyloestradiol, had no significant effect upon the extent of uterine contamination (Fig. 2).

Bromhexine hydrochloride significantly increased the extent of uterine contamination compared with vehicle control ($P<0·01$). Coadministration of prednisolone acetate, ethinyloestradiol or norethisterone acetate with bromhexine hydrochloride did not significantly alter the effect produced by administration of bromhexine hydrochloride alone (Fig. 3).

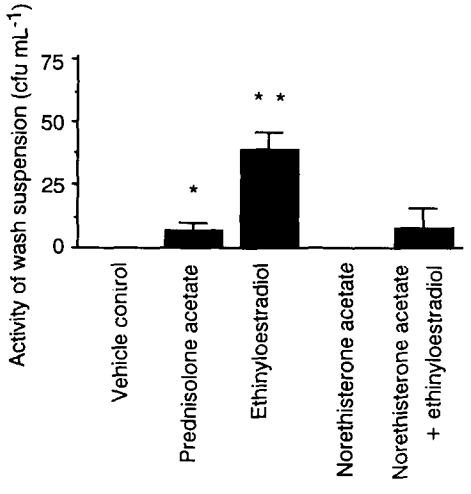

FIG. 2. Extent of uterine contamination in guinea-pigs receiving prednisolone acetate (2 mg kg^{-1}) and norethisterone acetate (1 mg kg^{-1}) either alone or in combination with ethinyloestradiol (0·05 mg kg^{-1}) or vehicle control daily for 21 days. *$P<0·05$, **$P<0·001$ compared with vehicle control.

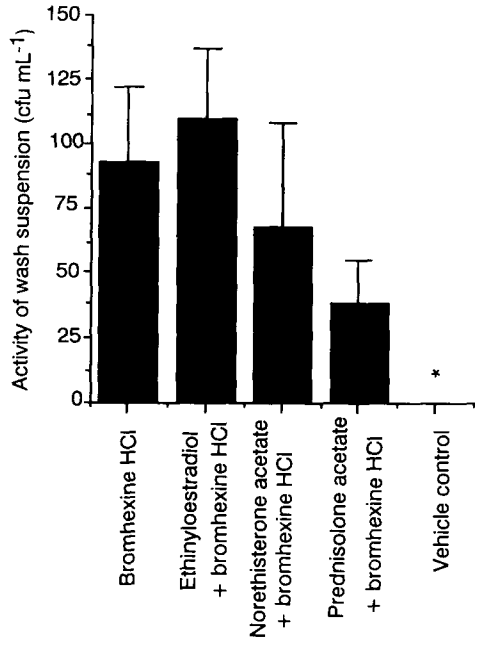

FIG. 3. Extent of uterine contamination in guinea-pigs receiving bromhexine HCl (30 mg kg^{-1}) either alone or in combination with prednisolone acetate (2 mg kg^{-1}), ethinyloestradiol (0·05 mg kg^{-1}) or norethisterone acetate (1 mg kg^{-1}), or vehicle control daily for 21 days. *$P<0·01$ compared with bromhexine HCl alone.

Discussion

All three of the steroid hormones induced a significant reduction in circulating white blood cell count, which may indicate some degree of immunosuppression. However, the extent of any immunosuppression is indeterminable. Bromhexine hydrochloride had no effect on the white blood cell counts, suggesting that its effect on the cellular immune system was negligible, although some drugs, for example cyclosporin, are potent immunosuppressants without producing significant effects on the total white blood cell count. There are, however, no reports of any immunosuppressant effects of bromhexine hydrochloride.

Ethinyloestradiol and norethisterone acetate caused white blood cell count reduction of a similar order of magnitude yet only ethinyloestradiol led to uterine contamination. Prednisolone acetate caused a much greater degree of leucopenia yet did not cause a significant increase in the incidence of uterine contamination and such a result suggests that leucopenia itself does not predispose to the colonization of the uterus by vaginal bacteria. If the degree of leucopenia is accepted as an index of steroid-induced immunosuppression then the results indicate that immunosuppression is unlikely to increase the incidence of microbial contamination of the uterus over the timescale studied.

Bacteria could be isolated from the uterus in all animals administered bromhexine hydrochloride orally (Malhi et al 1987) and in 6 out of 7 animals when administered intraperitoneally (this study). The most likely explanation of this drug's effect is that it reduces the viscoelasticity of cervical mucus and thus increases the likelihood of bacteria being able to breach the cervical mucus plug and enter the uterus. It can be postulated that the lack of bacterial migration during

the normal sex cycle is due to the short duration of low mucus viscoelasticity whereas in the presence of bromhexine hydrochloride the cervical mucus barrier is compromised for sustained periods.

A similar explanation may also pertain to the effects of ethinyloestradiol. Oestrogenic hormones such as ethinyloestradiol cause the secretion of this watery cervical mucus and thus the reduced mucus viscoelasticity may predispose to an increased microbial entry to the uterus. Progestogenic hormones such as norethisterone acetate are known to induce the secretion of highly viscid cervical mucus. Such cervical mucus would present a greater barrier to the transfer of bacteria. Hence the result for norethisterone acetate suggests that the thickened cervical mucus is able to maintain a sterile uterus even in the presence of immunosuppression. Further support for this suggestion comes from the finding that norethisterone acetate was able to reverse the effects of ethinyloestradiol on uterine microflora. There was no reversal of the leucopenia hence the antagonist effects probably depend upon the opposite effects of the two drugs on cervical mucus. Thus it appears that ethinyloestradiol predisposes to the entry of bacteria into the uterus by compromising the cervical mucus plug, although the coadministration of norethisterone acetate, which is known to increase the viscosity of cervical mucus, can reverse this effect at the doses used.

There are no previous reports of prednisolone acetate producing any effect on cervical mucus secretion. In the present study the incidence of uterine contamination in the prednisolone-treated group was not significantly greater than that for the vehicle control group, although it did lead to contamination in 3 of the 8 experimental animals. Whether a longer duration of prednisolone treatment would have increased the incidence of uterine contamination is unknown. The degree of contamination in those 3 animals affected was sufficiently high to produce a significantly higher extent of contamination in the prednisolone acetate treated group than in the vehicle control group. Prednisolone acetate also produced the most profound leucopenia of the drugs tested, hence these results indicate either that prednisolone has some slight oestrogenic effect upon cervical mucus or that profound leucopenia may predispose to uterine contamination. The former of these possibilities is the more likely as prednisolone acetate did not significantly increase the extent of uterine contamination induced by bromhexine hydrochloride; if the effects of prednisolone acetate were due to an immunosuppressive action, the combination of a mucolytic drug with an immunosuppressant might be expected to produce extensive uterine contamination.

Neither ethinyloestradiol nor norethisterone acetate were able to significantly influence the incidence or extent of uterine contamination induced by bromhexine hydrochloride, indicating either that the potencies of the steroid hormones are insignificant in comparison with the potency of bromhexine hydrochloride, or that the two classes of drug are acting at different stages of mucus synthesis and secretion. In the light of the extent of uterine contamination induced by ethinyloestradiol compared with the extent induced by bromhexine hydrochloride, the former suggestion appears to be the more likely.

This study indicates that the cervical mucus plug is more important than the cellular immune system in the maintenance of a sterile uterus. Thus drugs which decrease the viscoelasticity of the cervical mucus may predispose to uterine contamination, whilst drugs which increase mucus viscoelasticity may offer some protection. Ethinyloestradiol was found to cause an increase in the incidence and extent of uterine contamination; however, the magnitude of this effect was much less than the effect produced by bromhexine hydrochloride. The results also suggest that prednisolone acetate may also possess oestrogen-like actions on cervical mucus, and although these actions do not significantly increase the incidence of uterine contamination, the additional immunosuppressive actions of prednisolone acetate mean that the extent of any uterine contamination arising due to the hormone's action on cervical mucus may be greater.

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