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# The effects of bromhexine hydrochloride and *S*-carboxymethyl-L-cysteine on guinea-pig uterine microflora

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This study used guinea-pigs as a mammalian model to investigate the effects of bromhexine hydrochloride and *S*-carboxymethyl-L-cysteine on the integrity of the cervical mucus plug. It was shown that under normal circumstances the uterus is sterile, but following drug administration microorganisms began to appear in the uterus with no significant effect on the vaginal microbial population. It therefore appears that these two mucolytic agents may reduce cervical mucus viscoelasticity. After the animals had been mated, microorganisms were isolated from the uterus even in the absence of drug treatment.

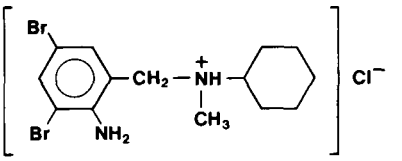
The normal microflora of the human vagina is subject to a complex array of hormonal and chemical interactions which result in variations with age and throughout the menstrual cycle. Quantitative determinations of the bacteriology of the vagina have revealed that anaerobic and facultative microorganisms are present in the highest concentrations per gram of swabbed material and include *Lactobacillus* spp., *Peptococcus* spp., *Bacteroides* spp., *Staphylococcus epidermidis, Corynebacterium* spp., *Peptostreptococcus* spp. and *Eubacterium* spp. (Bartlett et al 1977), and coliforms, *Candida albicans* and *enterococci* (Corbishley 1977; Watt et al 1981).

In contrast to the vagina, the microbial status of the uterus is difficult to determine but the most reliable information gained from hysterectomy patients has shown it to be sterile (Sparks et al 1977). The maintenance of sterility, in spite of the abundance of microorganisms within the vagina, is achieved due to a variety of factors including lysozyme secretions within the cervix, secretory IgA and cervical mucus viscoelasticity.

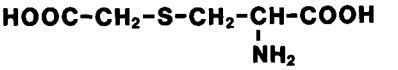
The cervix contains a plug of mucus, the properties of which vary cyclically throughout the menstrual cycle. In women, at the time of ovulation, the Spinnbarkeit and the water content of cervical mucus both increase with a corresponding decrease in viscoelasticity (Elstein 1978). During the luteal and follicular stages of the oestrous cycle the mucus produced is scanty, has high viscoelasticity and during these stages acts as a barrier to the penetration of sperm since penetration is inversely related to viscoelasticity (Elstein 1974; Kerin et al 1976).

Pelvic inflammatory disease (PID) is a general term referring to an acute or chronic infection of the uterus and fallopian tubes and occurs when bacteria gain entry to the uterus and multiply beyond the defence capabilities of the female genital tract. Several mechanisms by which the microbes may gain access to the uterus have been postulated. Keith et al (1984) suggested that bacteria may be able to attach to trichomonads or spermatozoa and hence utilize 'donor' motility to traverse the cervix. Alternatively, bacteria may undergo passive transport through the cervical mucus, presumably at the time of reduced mucus viscoelasticity, i.e. menstruation and ovulation. Similarly, the penetration of low viscoelasticity cervical mucus may be achieved by vigorously motile bacteria.

Using the guinea-pig as a mammalian model, we have examined normal vaginal and uterine microflora at different stages of the oestrous cycle and investigated the influence of mucolytic drug treatment on genital tract flora.




(BHC)




(SCMC)

Mucolytic agents such as bromhexine hydrochloride (BHC) and *S*-carboxymethyl-L-cysteine (SCMC), reduce mucus viscoelasticity and are used clinically in a variety of respiratory conditions which are associated with production of excessive amounts of highly viscoelastic mucus. The actions of these agents at other sites within the body have not been determined, but if cervical mucus viscoelasticity is reduced for extended periods of time then one of the main barriers to the entry of vaginal microorganisms into the uterus would be undermined.

Direct investigation into the effects of mucolytic agents on cervical mucus in animals is difficult to achieve owing to the very small quantities of material involved. An alternative approach is to use the presence or absence of vaginal bacteria in the uterus as an indication that the cervical mucus plug has been compromised.

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*Materials and methods*

*Animals.* Female Dunkin-Hartley guinea-pigs (300–500 g) were kept in groups of eight, in wire-bottomed cages (North Kent Plastic, Dartford, Kent).

*Determination of stage of oestrous cycle.* Twelve animals were studied for four oestrus cycles. Following lavage with a minimal amount of sterile, quarter-strength Ringer solution, microscopic examination of the resultant cell suspension allowed determination of the stage of cycle using the criteria of Stockard & Papanicolaou (1917). This procedure was repeated weekly during anoestrus and every 2 h during the shorter stages of the cycle.

*Determination of vaginal and uterine microflora.* Vaginal lavage was performed by repeatedly aspirating 2 mL sterile quarter-strength Ringer solution. The resultant suspension was appropriately diluted and 0·2 mL aliquots were plated onto blood agar plates which were incubated both aerobically and anaerobically at 37 °C for 24–48 h.

For the determination of uterine microflora, the uterus was dissected out under aseptic conditions and one horn was incubated whole in nutrient broth at 37 °C for 24–48 h. The lumen of the other horn was washed with sterile, quarter-strength Ringer solution and aliquots of 0·2 mL were plated onto blood agar plates and incubated as for the vaginal lavages.

After incubation the number of colonies per plate was determined and the number of colony forming units (cfu) per mL of original wash suspension calculated. Those microorganisms occurring in the greatest numbers were isolated and identified using standard techniques (Cowan 1974).

*Mucolytic drug treatment.* The mucolytic compounds used were bromhexine hydrochloride (BHC, Sigma Chemical Company, Poole) and *S*-carboxymethyl-L-cysteine (SCMC, Rorer Health Care, Eastbourne). The mucolytic was freely administered orally in a commercially available cereal base which also contained sucrose. The concentrations used were 0·1% w/w BHC and 1·0% w/w SCMC and these are approximately equivalent to 25 mg $kg^{-1}$ $day^{-1}$ of BHC and 300 mg $kg^{-1}$ $day^{-1}$ of SCMC.

Forty-eight guinea-pigs were used, divided into six groups: two groups acted as vehicle controls, two groups were given 0·1% BHC and two groups were given 1·0% SCMC. Seven days after the initiation of drug treatment the groups were further subdivided into those that were mated and those that were not, such that the six groups could be identified as follows: Group 1 unmated control, Group 2 unmated BHC, Group 3 unmated SCMC, Group 4 mated control, Group 5 mated BHC, Group 6 mated SCMC. Drug administration was then continued for a period exceeding one oestrous cycle at the end of which uteri were examined.

*Results*

The dominant phase of the guinea-pig oestrus cycle is anoestrus lasting for 15–17 days. The remaining stages are much shorter with oestrus lasting only 2–4 h. Vaginal washes were taken at different stages of the oestrus cycle and the resident microflora quantified. The results indicated that there were no statistically significant variations in the vaginal microflora during the oestrus cycle (Table 1).

Table 1. Results of studies on the microflora of guinea pig vagina. 12 untreated, unmated animals were observed for 3 oestrus cycles.

| | | Count (millions per mL of original wash), mean ± s.d. | |
|---|---|---|---|
| Stage | Duration | Aerobic | Anaerobic |
| Proestrus | 6–12 h | 460 ± 160 | 510 ± 180 |
| Oestrus | 2–4 h | 480 ± 160 | 430 ± 170 |
| Metoestrus | 4–6 h | 460 ± 170 | 550 ± 180 |
| Dioestrus | 1–2 h | 500 ± 285 | 430 ± 270 |
| Anoestrus | 15–17 days | 490 ± 150 | 400 ± 140 |

Those microorganisms occurring in the greatest numbers were isolated and identified and were found to be principally Enterobacteriaceae; in particular *Escherichia coli, Klebsiella* spp., *Proteus* spp. and *Enterobacter* spp. The same bacteria were isolated on both aerobically and anaerobically incubated media. No qualitative changes in vaginal microflora were evident during the oestrus cycle.

The vaginal aerobic and anaerobic microbial counts for animals receiving mucolytic drugs did not differ significantly from the counts for the control group. Similarly, mating had no significant effect on the vaginal microflora (Table 2).

Table 2. Vaginal microflora of unmated and mated groups. Results are given as mean ± s.d. (n = 8). Units colony forming units per millilitre of original wash solution × $10^6$.

| | Vaginal count | |
|---|---|---|
| Group | Aerobic | Anaerobic |
| Control (unmated) | 460 ± 30 | 475 ± 40 |
| BHC (unmated) | 490 ± 30 | 490 ± 50 |
| SCMC (unmated) | 480 ± 50 | 500 ± 60 |
| Control (mated) | 485 ± 50 | 470 ± 60 |
| BHC (mated) | 505 ± 50 | 470 ± 30 |
| SCMC (mated) | 500 ± 40 | 495 ± 30 |

The presence of bacteria in the uterus was determined by immersion of one horn in a suitable growth medium, and by washing, followed by plating from the other horn. In this way the presence of very small numbers of bacteria may be detected, even if they cannot be quantified. In each case where a count of zero was recorded there was no growth present in the horn when incubated whole. The unmated control group was shown to have uteri free from microorganisms as determined by both qualitative and quantitative exami-

Table 3. Number of organisms in the uteri of unmated and mated groups. Results are given as colony forming units per millilitre of original solution for each individual animal. Observations of growth (+) or no growth (−) from the uterine horn incubated whole are also given.

| | Unmated | | | | Mated | | | |
|---|---|---|---|---|---|---|---|---|
| Group | Animal | Whole Uterus | Uterine counts Aerobic | Anaerobic | Animal | Whole Uterus | Uterine counts Aerobic | Anaerobic |
| Control | 1 | − | 0 | 0 | 25 | + | 990 | 995 |
| | 2 | − | 0 | 0 | 26 | + | 745 | 1050 |
| | 3 | − | 0 | 0 | 27 | − | 0 | 0 |
| | 4 | − | 0 | 0 | 28 | + | 455 | 625 |
| | 5 | − | 0 | 0 | 29 | − | 0 | 0 |
| | 6 | − | 0 | 0 | 30 | − | 0 | 0 |
| | 7 | − | 0 | 0 | 31 | + | 785 | 1110 |
| | 8 | − | 0 | 0 | 32 | + | 1085 | 995 |
| BHC | 9 | + | 425 | 720 | 33 | + | 1170 | 1305 |
| | 10 | − | 0 | 0 | 34 | + | 980 | 795 |
| | 11 | + | 330 | 640 | 35 | + | 610 | 836 |
| | 12 | + | 750 | 785 | 36 | + | 870 | 710 |
| | 13 | + | 515 | 930 | 37 | + | 1095 | 1065 |
| | 14 | − | 0 | 0 | 38 | + | 320 | 265 |
| | 15 | − | 0 | 0 | 39 | − | 0 | 0 |
| | 16 | − | 0 | 0 | 40 | + | 845 | 935 |
| SCMC | 17 | − | 0 | 0 | 41 | + | 695 | 935 |
| | 18 | + | 275 | 380 | 42 | + | 440 | 600 |
| | 19 | + | 220 | 245 | 43 | − | 0 | 0 |
| | 20 | − | 0 | 0 | 44 | − | 0 | 0 |
| | 21 | + | 385 | 375 | 45 | + | 510 | 665 |
| | 22 | + | 170 | 235 | 46 | + | 575 | 525 |
| | 23 | − | 0 | 0 | 47 | + | 470 | 305 |
| | 24 | − | 0 | 0 | 48 | + | 445 | 495 |

Differences in bacterial presence between unmated control group and unmated, drug-treated groups were significant ($P < 0.05$ Fisher's Exact Test).

nation. Upon drug treatment, however, bacteria were isolated from approximately 50% of uteri, the organisms isolated being the same as those found in the vagina.

In those groups which were mated, microorganisms were found in approximately 75% of uteri including the drug-free control (Table 3).

*Discussion*

Bromhexine hydrochloride and *S*-carboxymethyl-L-cysteine are classified as mucolytic agents which have been shown to produce a decrease in the quantity and viscoelasticity of tracheal and bronchial mucus in both man (Gordon et al 1976; Aylward et al 1985) and animals (Richardson & Phipps 1978). Their administration is claimed to bring about a reduction in the viscoelasticity of mucus via a decrease in the synthesis and secretion of mucus rather than a direct alteration of mucus structure. However, their effects on mucus in other sites of the body have not been investigated, mainly due to the difficulties involved in obtaining samples of a suitable volume.

The results obtained in this study show that bacteria were able to migrate from the vagina into the uterus in those animals which were treated with mucolytic agents. Microorganisms were not detected in the uteri of any of the controls. This suggests that bacteria may breach the cervix under the influence of mucolytic agents, although our study does not allow elucidation of the mechanisms underlying this effect.

Since dosing of the animals in the drug-treated group was on an uncontrolled basis, the final dose of mucolytic received by each animal could not be monitored. This may, in part, account for the fact that not all of the animals in the drug-treated group exhibited microorganisms within the uterus. In the mated groups, one male was kept with eight females for a period exceeding one oestrus cycle. This experimental protocol was required since the period of oestrus only lasts for 2–4 h out of a total of 16–20 days and mating only occurs during oestrus. However, mating may not have occurred at all or it may have occurred immediately after the introduction of the male such that any contamination which was introduced was eliminated by the immune defences. Consequently, this may account for the isolated instances where no bacteria were recovered from the uterus.

The presence of microorganisms within the uterus after mating suggests that this is a natural occurrence not resulting in any overt infection. The mechanism by which bacteria enter via this route is unclear since it has been suggested that in guinea-pigs the site of sperm deposition is directly into the uterus (Handbook of Biological Data 1956). Bacteria could therefore be carried into the uterus as a result of the penis breaching the cervix. The bacterial counts obtained from the

mated animals were of a similar order of magnitude as the mucolytic treated animals and since infections do not normally arise as a result of mating, the level of contamination in the drug-treated groups could not be considered to be unusually high.

The results of this study suggest that the mucolytic agents bromhexine hydrochloride and *S*-carboxymethyl-L-cysteine compromise the integrity of the cervical mucus plug and that this is sufficient to allow the transmission of bacteria from the vagina into the uterus. If this effect also occurs in women there may be a variety of clinical implications. Mucolytic agents may be of use in the treatment of those cases of infertility which result from the presence of hyper-viscous, hostile cervical mucus. In addition, long term treatment with mucolytic agents may also predispose some women to pelvic infections due to a breakdown in the natural barrier between the uterus and vagina. The results also indicate that although the uterus is generally accepted as being a sterile environment, bacteria are recovered from it following mating in the guinea-pig.

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# Proglumide, a cholecystokinin receptor antagonist, exacerbates alloxan-induced diabetes mellitus in Swiss mice

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The effect of proglumide ((±)-4-benzamido-*N,N*-dipropylglutaramic acid), a gastrin and cholecystokinin receptor antagonist, has been studied on the fasting plasma glucose (FPG) and insulin levels in normal and alloxan-diabetic mice. In normal mice, proglumide, administered as a single oral dose or twice daily for five consecutive days, did not produce any alteration in those parameters. Injection of alloxan monohydrate (70 mg $kg^{-1}$ i.v.) produced a significant decrease in plasma insulin and a significant elevation of FPG levels on the 5th day after its administration as evidence of diabetes mellitus. Proglumide sodium, given as a single acute dose on the 5th day of alloxan injection, or as a twice daily dose for 5 days immediately after alloxan injection, significantly exacerbated the hyperglycaemia and further decreased the plasma insulin levels thus worsening the diabetogenic effect of alloxan. These observations point to a possible involvement of cholecystokinin (CCK) in alloxan-induced diabetes and indicate a need for monitoring the levels of FPG in diabetic patients being treated with a high dose of proglumide or other CCK-antagonists.

Proglumide ((±)-4-benzamido-*N,N*-dipropylglutaramic acid) has been shown to block gastrin receptors (Chiodo & Bunney 1983; Magous & Bali 1983), reduce gastric acid secretion (Rovati 1976), increase gastric mucosal resistance (Weiss 1979) and to possess significant anti-ulcer activity in animals (Umetsu et al 1980; Parmar 1986; Tariq et al 1987) and man (Galeone et al 1979). Niederaus et al (1985) found it to be significantly effective against the caerulein-induced acute necrotizing pancreatitis in mice. It is structurally related to the C-terminal tetrapeptide amide of both gastrin and cholecystokinin (CCK) and thus it inhibits the binding of $^{125}$I-labelled CCK to its receptors in the pancreatic acini (Hahne et al 1981; Williams et al 1983). CCK stimulates insulin release from the endocrine pancreas (Szecowka et al 1982) and specific CCK receptors have been demonstrated in rat isolated pancreatic islets (Verspohl et al 1986a). Recently, Verspohl et al (1986b), using fresh and cultured rat isolated islets of Langerhans, have shown that proglumide also produces a CCK-dependent inhibitory effect on insulin secretion in-vitro. Their data indicate that CCK antagonists should be monitored for a possible diabetogenic effect in-vivo. The present investigation was therefore undertaken to study the effect of proglumide on the fasting plasma glucose and insulin levels in normal and alloxan-induced diabetic mice.

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