Antigonococcal effects of vaginal tampons

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SUMMARY Different brands of vaginal tampons varied significantly ($p < 0.0001$) in their antibacterial effects when tested with 46 strains of *Neisseria gonorrhoeae*. Gonococcal strains recovered from patients with disseminated infections were substantially more sensitive to the antibacterial effects of tampons than were strains from patients with uncomplicated genital infections. Strains from patients with pelvic inflammatory disease were moderately sensitive. Tampons showing strong in-vitro antigonococcal effects were also generally effective in vivo in eliminating gonococcal infections from subcutaneous chambers in mice. Extracts of the Rely tampon showed no in-vitro antigonococcal effect, however, but did induce antibacterial activity when injected into subcutaneous chambers in mice. These results emphasise the importance of both in-vitro as well as in-vivo testing of tampon materials to elucidate more fully the nature of their antibacterial effects and their potential for affecting vaginal pathogens and disease processes.

Introduction

Since the introduction of vaginal tampons more than 45 years ago, the variety of products and complexity of materials used in their manufacture have steadily increased. Consequently, the effects of tampon components on vaginal micro-organisms and on the immunological responses of women using them are causing increasing concern. The recent association of *Staphylococcus aureus* and the use of superabsorbent tampons with increased risk for developing toxic shock syndrome (TSS) in menstruating women has stimulated interest in the biological effects of tampon components.^{1,2}

In earlier studies we tested the Rely* and Tampax Super Plus Tampons, as well as some components used in tampon manufacture, for biological effects with a strain of *Neisseria gonorrhoeae* isolated from the joint fluid of a patient with disseminated infection. Significant differences were found between different kinds of tampons and tampon components in their inhibitory effect on gonococci and their ability to affect the dissemination of gonococci from the peritoneal cavity of mucin-treated mice.³ The present study has extended these previous findings to

include tests of 16 different commercial tampons with 46 strains of *N gonorrhoeae* isolated from patients who had (a) disseminated gonococcal infection, (b) pelvic inflammatory disease, or (c) uncomplicated gonococcal infection. In addition, aqueous extracts of selected tampons were tested for their ability to alter the resistance of mice to subcutaneous chamber infections with *N gonorrhoeae*. The results of these experiments are reported.

Materials and methods

TAMPONS

Eighteen different brand-named vaginal tampons (table I) were purchased from stores in Atlanta, Georgia.

BACTERIA AND IN-VITRO TESTS

Strains of *N gonorrhoeae* from patients in various areas of the United States and acquired by the sexually transmitted diseases (STD) laboratory at the Centers for Disease Control, Atlanta, Georgia, which had been recently isolated and identified by standard laboratory tests were selected for study. A suspension of cells (about 10^8 cfu/ml) from each strain was prepared in sterile water containing 5% monosodium glutamate and 5% bovine serum albumin. Aliquots of each strain (0.5 ml) were stored in glass vials frozen at -55°C until thawed for inoculation on to a growth medium. The strains included 18 isolates from patients with disseminated infection (DGI), 16 isolates from women with pelvic

*Use of trade names is for identification only and does not imply endorsement by the Public Health Service or by the US Department of Health and Human Services.

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TABLE 1 Percentage of *Neisseria gonorrhoeae* strains inhibited by tampons*

Tampon	Lot	DGI strains (%)	PID strains (%)	NGI strains (%)
Rely	1550LA16A	0	0	0
Tampax Regular	T4121	22	6	0
Tampax Super	T375T	22	6	0
OB Regular	NA	28	6	0
OB Super	NA	44	6	0
Kotex Regular	A2C2670B	47	19	23
Kotex Super	A3C580B	47	24	15
Playtex Super				
Deodorant	011516	72	50	3
Revco Regular	0H1115	77	19	8
Tampax Super Plus	6T072T	78	69	15
Playtex Super Plus				
Deodorant	0K1111	83	61	63
Pursettes	27091	88	19	23
Playtex Regular	0H714	89	48	47
Playtex Super Plus	0J151826	94	38	31
Playtex Regular				
Deodorant	01262	94	88	23
Playtex Super	0B280329	100	91	84

*Number of strains tested with tampons were: 17 from disseminated infections (DGI), 16 from pelvic inflammatory disease (PID), and 13 from uncomplicated genital infections (NGI).

inflammatory disease (PID), and 13 isolates from patients with uncomplicated genital infection (NGI). For in-vitro testing plates of GC base supplemented with 1% IsoVitalX (BBL Microbiology Systems, Cockeysville, MD) were seeded with 10^5 cfu of *N. gonorrhoeae* suspended in 0.05 ml of phosphate-buffered (0.06 mol/l KH_2PO_4 and Na_2HPO_4) saline (PBS). A glass rod was used to spread the suspension over the agar surface. Tampons were cut into pieces of about 20 mg each and those from different tampons were arranged in the plate contacting the seeded agar surface. Six to 10 pieces were usually tested on each 100-mm plate. After incubation in a candle-extinction jar for 20 hours, the plates were examined and the zones of inhibition extending from the edge of each tampon piece measured (mm).

IN-VIVO TESTS

Three kinds of tampons which showed in-vitro inhibitory effects and three which did not, as tested with *N. gonorrhoeae* strain 47 joint, were selected for study in vivo by a subcutaneous chamber model of gonococcal infection.³⁻⁵ The tampons were placed in 50 ml of PBS, pH 7.4, and agitated on a rotary platform at 90 rev/min. After extraction for 30 minutes 15 ml of fluid was expressed from each tampon. Extracts from which no bacterial growth was obtained after inoculation on to a GC base medium were then used for injection. For the in-vivo tests female Institute of Cancer Research (ICR) strain white mice weighing >25 g were surgically implanted

with porous stainless steel cylindrical chambers (1 × 2 cm). After 7 to 10 days to allow their incisions to heal the mice were randomly placed in seven groups of 20-31 mice and injected intrachamberly with 0.5 ml of tampon extract. Control animals received 0.5 ml of PBS. Thirty minutes later each mouse was challenged with approximately $10^{3.7}$ cfu of gonococci strain 47 joint. The challenge dose was suspended in 0.1 ml trypticase soy broth and was equivalent to about 100 chamber 50% infectious doses (CID_{50}). Their infection status was tested by aspirating an aliquot of 0.05 ml of chamber fluid from each mouse 24 hours after challenge, streaking this on to GC base, and incubating as before. The inoculated plates were examined the next day for growth of gonococci. Mice with positive cultures from their chamber fluid were removed from the various groups, pooled, and randomly regrouped for use in the following experiment to determine the efficacy of tampon extracts for inhibiting established infections.

The mouse chambers which had been infected with gonococci for 24 hours or longer were injected with 0.5 ml of different tampon extracts or with PBS at 9 am and 3 pm for two consecutive days and again at 9 am on the third day (figure). Chamber fluid specimens were obtained from each mouse at 8 am on days 1, 2, 3, and 6 to determine the infection status of the chamber.

ANALYSIS OF DATA

The inhibitory zone (mm) for each tampon tested with 47 gonococcal strains was measured visually. A computed statistical analysis using the Kruskal-Wallis test (χ^2 approximation) was performed to

FIGURE Effects of tampon extracts on the elimination of *Neisseria gonorrhoeae* from subcutaneous chambers in mice. (A: Tampax Super Plus; B: Playtex Super; C: Playtex Super Deodorant; D: Rely; E: Tampax Regular; F: Kotex Super; G: phosphate-buffered saline control.) Number of chamber fluid cultures made on days 0, 1, 2, 3, and 6 over number tested are given in parentheses. Arrows indicate times of 0.5-ml intrachamber injections of aqueous tampon extracts.

compare the effects of various types and brands of tampons on the inhibition of gonococci. Data for in-vivo tests of six tampons (figure) were analysed by the χ^2 test.⁵

Results

Tampons varied significantly ($p < 0.0001$) in their in-vitro inhibition of gonococci (table I). Playtex tampons inhibited the greatest number of gonococcal strains while Rely tampons, which contained cross-linked derivative of carboxymethyl cellulose (CLD-CMC), did not show any inhibitory activity with the 46 strains tested. Significant ($p < 0.01$) variations were observed among different types of Playtex and Tampax tampons when they were tested with the same strains of gonococci (table I). Deodorant Playtex did not appear significantly ($p > 0.05$) different from non-deodorant Playtex tampons in the number of gonococcal strains inhibited or the zone size of inhibition. For most tampons tested the percentage of strains inhibited and the zone size of inhibition appeared to be directly related. DGI strains were substantially more sensitive to in-vitro inhibition by tampons than were gonococci from uncomplicated genital infections. PID strains were intermediately sensitive (tables I and II).

Results for the in-vitro and in-vivo tests generally agreed; however, the Rely tampon which showed no in-vitro antigonococcal activity significantly increased the resistance of mice to subcutaneous chamber infections when extract of the tampon was injected 30 minutes before challenge. As shown in

TABLE II Median zone of inhibition (mm) produced by tampon materials reacted with *Neisseria gonorrhoeae* strains*

Tampon	Lot	DGI strains	PID strains	NGI strains
Rely	1550LA16A	0.0	0.0	0.0
Tampax Regular	T4121	0.3	0.0	0.0
Tampax Super	T375T	0.2	0.0	0.1
OB Regular	NA	0.3	0.0	0.0
OB Super	NA	0.8	0.5	0.0
Kotex Regular	A2C2670B	0.9	0.8	0.2
Kotex Super	A3C580B	1.2	0.8	0.2
Playtex Super				
Deodorant	011516	3.6	1.5	0.5
Revco Regular	0H1115	3.2	0.8	0.2
Tampax Super Plus	6T072T	3.3	1.4	0.2
Playtex Super Plus				
Deodorant	0K1111	4.0	1.5	1.0
Pursettes	27091	2.8	0.8	0.3
Playtex Regular	0H714	3.2	1.6	1.1
Playtex Super Plus	0J151826	4.0	1.3	0.5
Playtex Regular				
Deodorant	01262	5.4	2.8	1.9
Playtex Super	0B280329	5.2	3.4	2.7

*Number of strains tested with each tampon were: 17 from disseminated infections (DGI), 16 from pelvic inflammatory disease (PID), and 13 from noncomplicated genital infections (NGI).

TABLE III Effects of tampon extracts on resistance of mice to infection with *Neisseria gonorrhoeae*

Tampon extract (group)	No infected	No challenged	% Infected	Pro-bability*
Tampax Super Plus (rayon 8285) (A)	18	22	81.8	0.069
Playtex Super (B)	17	21	80.9	0.154
Playtex Super Deodorant (C)	17	21	80.9	0.154
Rely (CLD-CMC) (D)	18	31	58.0	
Tampax Regular (E)	18	20	90.0	0.033
Kotex Super (F)	20	21	95.2	0.008
Phosphate-buffered saline (PBS) control (G)	19	21	90.4	0.026

* χ^2 probability compared with group D.

table III only 58% of mice receiving Rely extract and then challenged with 100 CID₅₀ of *N. gonorrhoeae* were culture-positive the following day. This infection rate was significantly ($p = 0.026$) lower than for mice receiving PBS or extracts of two other relatively non-inhibitory tampons ($p = 0.033$ and 0.008). The infection rates for mice receiving Rely extracts were not significantly ($p > 0.05$) different, however, from those of mice receiving extracts of tampons A, B, and C (table III), which showed in-vitro inhibition of gonococci. In tests to determine the effects of tampon extracts on established gonococcal infections the Rely extract induced strong in-vivo antigonococcal activity during the first day after injection (figure). The effectiveness of the Rely extract for eliminating established infections declined substantially, however, after the first day, and by the sixth day of the experiment 50% of the mice remained infected (figure). In comparison infection rates for groups of mice receiving extract from Tampax Super Plus, Playtex Super, or Playtex Super Deodorant tampons ranged from 0 to 44.4% on the sixth day. Extract from either Tampax Regular or Kotex Super had little in-vitro or in-vivo effect on *N. gonorrhoeae* (table I and figure). On rechallenge of previously infected mice 4-6 days after elimination of their first infection 90.4% were susceptible to re-infections with gonococci, indicating that elimination of the first infection after the injection of tampon extracts was not due to the development of immunity.

Discussion

The finding that certain tampons showed little in-vitro inhibition of gonococci but did induce significant inhibitory effects in vivo suggests the presence of host-reactive substance(s). Although the duration or the mechanism for the resistance induced in mice by tampon extracts were not specifically determined, non-specific stimulation of natural host defences

may have been involved. The longer-term effects of tampon materials on the host's cellular and humoral immune responses remain to be determined.

Although the practical implications for the anti-gonococcal effects of certain tampons are not known, Lossick *et al*⁷ reported in a preliminary study of tampon usage that women with gonococcal PID used tampons as frequently as women who did not develop PID. The present study shows, however, that the antimicrobial effects of different tampons may vary significantly. Therefore, a more detailed brand-by-brand analysis of tampon usage may be required to determine if significant differences exist in the frequency of complications of PID and DGI in women using different tampons. Lossick *et al*⁷ attempted to link tampons with an increased incidence of PID because of the possible reflux of infectious secretions into the uterus due to blockage of normal menstrual flow. Our data suggest that certain tampons may have antimicrobial effects that could influence gonococcal infections *in vivo*.

The findings that certain tampons had strong anti-gonococcal activities may also help to explain the results of some earlier studies. In studies to determine the efficacy of using tampons as a source of self-obtained culture specimens various results have been reported. In a study of 504 college women no positive cultures were obtained, while in another study with 297 women in a high-risk group 35.4% of tampon specimens were positive for *N gonorrhoeae*.^{8,9} In another study of 100 women, from whom both endocervical swabs and self-inserted tampons were obtained for culture, about the same number of women had positive results for both specimens if the tampons were smeared on to a growth medium immediately after removal from the vagina. Delays of one hour or longer after removal seriously reduced the number of culture-positive tampon specimens.¹⁰ The tampons used in the two studies^{8,10} were Tampax Junior. The fibre composition and any inhibitory effects of these tampons are not known. Although other differences between these studies may have influenced the results, the antimicrobial effects of the tampons used could have affected the sensitivity of the culture techniques.

Tampons may vary substantially in the relative composition of different fibres used in their manufacture. Therefore, observations in the present study for various brands reflect the composition and chemical processes used during the manufacture of these lots. Until the inhibitory nature of specific materials used in each tampon is known, tampons being considered for use in culture-related studies should be evaluated for possible inhibitory effects. Further studies are needed to elucidate more fully the potential benefits or hazards that may derive from tampons made of different materials. In addition, the inhibitory substances found in certain tampons need further characterisation to establish their mode(s) of action and their potential for affecting gonococcal as well as other vaginal infections.

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