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A modified transmembrane migration method for evaluating the spermicidal potency of some nonoxynol compounds

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Abstract—The transmembrane migration technique, a simple method in-vitro for quantitatively assessing the effects of a drug on human sperm motility, has been evaluated. The original method has been modified to include a preincubation step, and the incubation time has been reduced to 90 min. In semen samples possessing sperm concentrations of $< 75 \times 12^6$ spermatozoa mL^{-1} the volume of the lower reservoir has been reduced to 1 mL. This modified method has been used to compare the spermicidal potency of the widely employed non-ionic surfactant nonoxynol-9, with nonoxynol-5 and nonoxynol-15 (containing, respectively, fewer and more ethylene oxide units per molecule). The rank order of spermicidal potency of the compounds evaluated was nonoxynol-9 = nonoxynol-5 > nonoxynol-15.

The transmembrane migration method for measuring drug effects on human sperm motility (Hong et al 1981, 1983) is a simple in-vitro technique that requires only small volumes of semen, thereby facilitating the performance of multiple comparisons on a single semen sample. We have examined various aspects of this technique, and used a modified system for our evaluations of several nonoxynol compounds.

The most popular currently used vaginal contraceptives contain, as their active ingredient, the non-ionic surfactant nonoxynol-9 (Antarox or nonylphenoxypoly(ethylenoxy)ethanol), which produces its spermicidal action by causing damage to the cell membranes of sperm rendering them immotile and unable to penetrate the ovum (Wilborn et al 1983). Nonoxynol-9 is one member of a series of compounds with the average number of ethylene oxide units per molecule being indicated by the number. We have been unable to find any comparative data on the spermicidal activity of nonoxynols, and therefore undertook these comparisons of nonoxynol-9 with two other water-soluble nonoxynols: nonoxynol-5 and nonoxynol-15.

Methods

Nonoxynols were purchased from the GAF Corporation. The apparatus used for measuring the transmembrane migration of sperm was constructed essentially as described by Hong et al (1981, 1983) and comprises two chambers separated by a membrane (Nuclepore Corporation). The proportion of sperm which moves across the membrane from the upper to the lower chamber is assessed and expressed as the transmembrane migration ratio (TMR). Semen samples were collected by masturbation after at least three days' sexual abstinence, from a pool of eight healthy volunteers (18–35 years old, with an average age of 22). Samples, which were only viable for use up to 4 h after collection, were kept at room temperature (20°C) and used after liquefaction.

The characteristics noted of each sample included spermatozoa concentration and sperm motility, the percentage dead sperm being assessed using ethidium bromide and fluorescence microscopy. Only samples with more than 40×10^6 mL^{-1} spermatozoa and 40% motility were studied. 100 μL of semen (previously mixed 2:1 with phosphate-buffered saline (PBS) at pH 7·3, or drug dissolved in PBS) preincubated at 37°C for 10 min, were pipetted into the upper chamber. This chamber was then inserted into the lower chamber containing 2 mL phosphate-buffered saline at 37°C, and the apparatus then incubated in a water bath for 90 min at 37°C. The upper chamber was then removed and its contents washed into 2 mL phosphate-buffered saline. The sperm in each chamber were killed with 50 μL of 10% formalin and the number of sperm present in each chamber counted in a haemocytometer (modified Neubauer). By examining the various experimental conditions, we have attempted to optimize the method.

The data relating to the spermicidal experiments were analysed using an iterative, non-linear regression analysis programme (PCNONLIN, Statistical Consultants Inc. Lexington Ky, USA) to the parameters of the following equation:

$$I = I_0 \frac{I_0 C^s}{Q^s + C^s}$$

Where I_0 is the percentage of inhibited motility at 0 concentration of inhibitor, C is the variable drug concentration, Q (IC50) is the drug concentration at which 50% maximal inhibition occurs and S is a parameter controlling 'sigmoidicity' of the response curve.

Results

Evaluation of the technique. The experimental conditions evaluated are described below. We have included a preincubation step in order to prevent dilution of drug into the lower chamber which might otherwise result in the underestimation of its spermicidal potency.

Membrane characteristics. The TMR of semen mixed with phosphate-buffered saline alone was compared using three types of 5 μm pore size polycarbonate membranes; standard and chemotactic membranes with the standard wetting agent, non-toxic polyvinyl polymer (PVP) present or absent. The highest TMR value and lowest variability were obtained using the standard membranes ($35{\cdot}7 \pm 1{\cdot}1\%$ vs $21{\cdot}6 \pm 7{\cdot}4\%$ and $27{\cdot}6 \pm 4{\cdot}7\%$, respectively, for the two types of chemotactic membrane).

Sexual abstinence. Abstinence led to an increase in ejaculate volume (40%), total number of spermatozoa per ejaculate (57%) and concentration of spermatozoa (47%).

Incubation time. Investigation of the effects of incubation time on the TMR showed that a steady state was reached in the relative disposition of spermatazoa between the chambers after 30–60 min incubation.

Volume of the lower chamber. A study of the effects of the TMR of varying the volume of the lower chamber was undertaken because semen specimens containing $< 75 \times 10^6$ spermatozoa mL^{-1} showed an increased error of counting, owing to fewer sperm being present in the lower chamber. The coefficient of variation for samples with low sperm counts was 20%, compared with 7·5% for semen specimens containing $> 100 \times 10^6$ spermatozoa mL^{-1}. Variation in the volume of the lower chamber from 1 to 3·5 mL had no effect on the TMR.

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Variation in pH. The effect of pH on sperm motility was investigated either by varying the pH of the buffer mixed with semen before preincubation (the buffer of the lower chamber remaining at pH 7·3); or varying the pH of the contents of both chambers simultaneously. Neither treatment had any effect on the TMR over the pH range 5–9.

Spermicidal effects of nonoxynols. The dose-dependent inhibition of sperm motility produced by nonoxynols-5, -9 and -15 is shown in Fig. 1. The concentrations which reduce sperm motility to 50% (IC50) were $0{\cdot}11 \pm 0{\cdot}01$, $0{\cdot}10 \pm 0{\cdot}03$ and $0{\cdot}50 \pm 0{\cdot}02$ mg mL^{-1}, respectively.

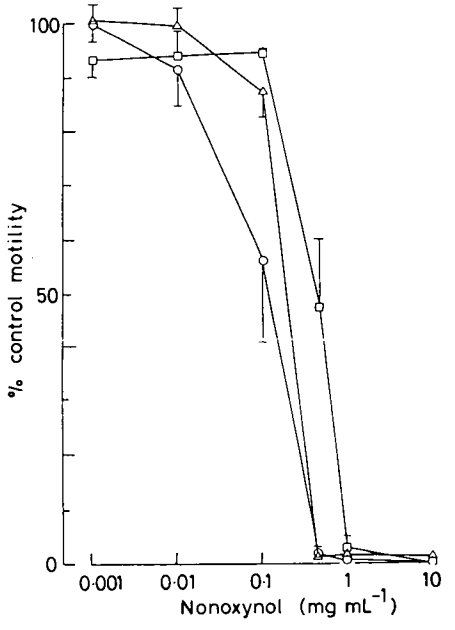

FIG. 1. Dose dependent inhibition of human sperm motility by nonoxynol-5 (○, n = 4), -9 (△, n = 7) and -15 (□, n = 4). Results are mean and standard error of n determinations performed in triplicate on ejaculates from different donors.

The dose response curves for nonoxynol-5 and nonoxynol-9 converge at approximately 0·5 mg mL^{-1}, when more than 98% inhibition of sperm motility is observed. The rank order of potency of the nonoxynols examined is therefore nonoxynol-5 = nonoxynol-9 > nonoxynol-15.

Discussion

Our observations of increasing ejaculate volume and of sperm concentration associated with sexual abstinence are compatible with those of Hargreave & Nilsson (1983).

The technique used is a simple means of assessing the effect of a drug on sperm motility. The TMR is dependent upon the characteristics of the membrane separating the upper and lower chambers, the standard polycarbonate membrane being the most efficient. The basic method developed by Hong et al (1981, 1983) has been modified to include a preincubation step aimed at reducing the possible underestimation of spermicidal activity of a compound. When semen samples have a sperm concentration of $< 75 \times 10^6$ spermatozoa mL^{-1}, the volume of the lower chamber is systematically reduced to 1 mL to minimize counting errors (this does not alter the TMR). It was observed that semen has a very high buffering capacity over the range examined (pH 5–9).

The IC50 value we have obtained for the effect of nonoxynol-9 on sperm motility is consistent with results obtained previously with the same technique (Hong et al 1983; Louis & Pearson 1985).

The differing potency of the nonoxynols with respect to their IC50 values corresponds to the relative lipophilic nature of each compound. There was observed an increase in the IC50 corresponding to an increase in side-chain lengths: 5 > 9 > 15. This suggests the importance of lipophilicity in the properties of these surfactants to diffuse into the sperm plasma membrane and perturb its conformation and semipermeable nature, preventing the occurrence of both sperm motility and ovum fertilization.

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