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Comparative effects of (+)-propranolol and nonoxynol-9 on human sperm motility in-vitro

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Abstract—Using the modified transmembrane migration method to measure sperm motility, it was shown that the surfactant nonoxynol-9 alone, was twice as potent as (+)-propranolol alone as a spermicidal agent. Addition of (+)-propranolol to nonoxynol-9 shifted the dose-response curve to the left of the curves for either component alone, and a surprising synergistic action was evident. These observations may form the basis for the development of a new advantageous topical contraceptive combination product.

The most effective spermicidal agents presently available are detergents or surfactants, and the most widely-used is nonylpolyethoxyethanol (nonoxynol-9) (Louis & Pearson 1985; Chijioke & Pearson 1986; Sharman et al 1986). Detergents diffuse into the sperm plasma membrane, perturbing its conformation and causing destruction of its semi-permeable nature, preventing the occurrence of both motility and fertilization (Schill & Wolf 1981; Wilburn et al 1983). Another group of compounds, the so-called membrane stabilizing agents, also inhibit sperm motility by exerting a non-specific action on membranes, including local anaesthetic or quinidine-like activity, physical stabilization of membranes and protection against cell lysis (Smith 1982). The β-adrenoceptor antagonist, propranolol, in addition to its specific therapeutic action in cardiovascular disease, and at concentrations in the millimolar range, displays membrane stabilizing activity and inhibits sperm motility (Peterson & Freund 1973; Curtis-Prior & Gadd 1990). Interestingly, the racemic mixture and both isomers of propranolol possess non-specific membrane effects and exhibit spermicidal activities, but the (+)-isomer is only a weak β-adrenoceptor antagonist (Barrett & Cullum 1968), so we have investigated the effects of (+)-propranolol and nonoxynol-9, alone and in combination, on human sperm motility, in-vitro.

Methods

The effects on sperm motility of nonoxynol-9 and (+)-propranolol were measured using the modified transmembrane migration method of Hong et al (1981), as previously described (Gadd & Curtis-Prior 1988), which allows dose-response curves to be performed on a single semen sample. Initially, dose-response curves were constructed on each ejaculate, from a pool of seven donors, with triplicate measurements of each concentration. Subsequently, more detailed data, covering a wide range of drug concentrations (with a single measurement of sperm motility at each) to define the shape of the curve more accurately, were analysed using the PCONLIN computer programme. First, the inhibitory activity of (+)-propranolol alone was investigated, then nonoxynol-9 alone, and finally nonoxynol-9 in combination with 1 and 2·5 mM (+)-propranolol on sperm samples from donors.

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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 inhibition of motility at zero 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 and discussion

Fig. 1 shows the mean effects of (+)-propranolol alone, nonoxynol-9 alone, and nonoxynol-9 in combination with 1 and 2·5 mM (+)-propranolol on the motility of human sperm from at least five donors. Nonoxynol-9 was shown to be approximately twice as potent as (+)-propranolol, their IC50 values being 0·156 mg mL^{-1} (0·106–0·207, 95% confidence interval) and 0·373 mg mL^{-1} (0·323–0·426), respectively. The addition of 1 or 2·5 mM (+)-propranolol to the nonoxynol-9 shifted the dose-response curve to the left of the curves for either agent alone, and

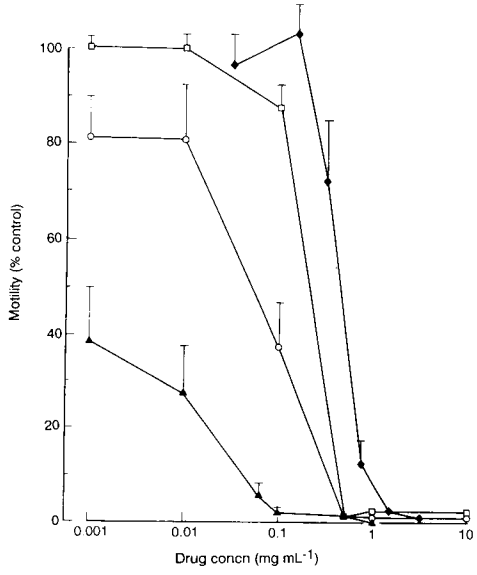

FIG. 1. Dose-response curves for the mean effects of (+)-propranolol, (◆) (n = 5); nonoxynol-9, (□) (n = 7); nonoxynol-9 plus 1 mM (0·3 mg mL^{-1}) propranolol, (○) (n = 5) and nonoxynol-9 plus 2·5 mM (0·75 mg mL^{-1}) propranolol (▲) (n = 5) on the motility of human sperm, in-vitro using the modified transmembrane migration method described by Gadd & Curtis-Prior (1988); (n) indicates the number of sperm donors involved. Nonoxynol-9 is approximately twice as potent as (+)-propranolol, but the addition of (+)-propranolol to nonoxynol-9 shifted the dose-response curve to the left of the curves for the individual components, indicating a synergistic action of the two drugs in inhibiting sperm motility.

Table 1. IC50 values for (+)-propranolol alone and nonoxynol-9 alone, and in combination with various concentrations of (+)-propranolol.

Donor	Nonoxynol-9	(+)-propranolol	Nonoxynol-9 + (+)-propranolol (1mM)	Nonoxynol-9 + (+)-propranolol (2·5mM)
1	0·435* (0·393–0·477)†	0·752 (0·693–0·811)	0·136 (0·125–0·148)	0·00479 (−0·0002–0·00983)
2	0·271 (0·225–0·316)	0·796 (0·684–0·909)	0·0516 (0·0369–0·0663)	0·0295 (0·0085–0·0505)

* Estimates are IC50 means from ejaculates from specific donors taken on three occasions and examined in triplicate.

† Figures in parenthesis are 95% confidence intervals of the estimate.

Table 2. Effects on percentage inhibition of nonoxynol-9 alone, (+)-propranolol alone, and in various combinations, on motility of sperm obtained from donor 3.

Conditions	Concn. (mM)	Inhibition of sperm mobility (%)
(a) Nonoxynol-9	0·16	12·5**
(b) Nonoxynol-9	1·60	97·7
(c) (+)-Propranolol	1·00	28·0
(d) (+)-Propranolol	2·50	76·5
(e) (a)+(c)		99·0
(f) (b)+(d)		99·9

** Estimates are derived from triplicate observations on ejaculates obtained on three occasions.

thus a surprising synergistic action of the two drugs in inhibiting sperm motility was indicated.

In further experiments, the inhibitory action of a combination of nonoxynol-9 and (+)-propranolol on sperm motility of four subjects was studied in detail and those data were analysed by a simple effect model. Reasonable agreement was found between the observed data and the model predictions, and using parameters derived from the model, the interaction between nonoxynol-9 and (+)-propranolol was explored. There was between three and ten fold increase in potency of nonoxynol-9 and 1 mM (+)-propranol compared with nonoxynol-9 alone (Table 1). This result was considered as synergistic, since the results were poorly explained by two models of non-synergistic interaction (Weinberg 1986), namely a "simple independent action" and "simple similar action". There appeared to be no further advantage in inhibiting sperm motility by increasing the dose of (+)-propranolol in combination with nonoxynol-9 above 1 mM, because although the IC50 value was decreased, the concentration of nonoxynol-9 causing total inhibition of sperm motility (approximately 0·5 mg mL^{-1}) was similar. The synergistic action of nonoxynol-9 and (+)-propranolol was evident also in another series of experiments (Table 2) when nonoxynol-9 (0·16 mM) alone caused 12·5% inhibition, (+)-propranolol (1·0 mM) alone caused 28% inhibition and in combination the two compounds at these same individual concentrations achieved 99% inhibition.

On the basis of these results appropriate concentrations of each component may be defined as nonoxynol-9 0·5 mg mL^{-1} and (+)-propranolol 0·3 mg mL^{-1} (approximately 1 mM), and enable the effective use of approximately one-tenth of the nonoxynol-9 employed in current contraceptive formulations, so reducing considerably the potential problems of sensitivity to this surfactant. Further, it may be relevant that propranolol has been shown to concentrate in cervical-vaginal mucus following oral administration to healthy female volunteers (Pearson et al 1985). Our observations may form the basis, therefore, for the development of a novelly advantageous topical contraceptive combination product.

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