A modified trans-membrane migration method for measuring the effect of drugs on sperm motility

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- 1 The trans-membrane migration ratio (TMMR) technique is a simple method for quantitating and comparing the effect of drugs on sperm motility.
- 2 The ultrafilter membrane (Nucleopore) used allows mixing between the solutions on either side resulting in 21-fold dilution of a drug initially mixed with semen.
- 3 The concentration of drug originally mixed with semen does not remain constant over the incubation time.
- 4 The phosphate buffer saline (PBS) in the lower chamber should be replaced by PBS containing the same concentration of drug as in the upper chamber so that a homogenous system is established throughout the incubation.
- 5 Reports of previous studies using this technique may have underestimated the effect of drugs on sperm motility.

Keywords sperm motility semen

Introduction

Testing the effect of drugs on sperm motility is of importance in screening drugs when attempting to find novel, effective spermicidal agents and drugs which enhance sperm motility. The trans-membrane migration ratio (TMMR) method has been used to measure the effect of various pharmacological agents on sperm motility (Hong *et al.*, 1981a). It is objective, simple, quantitative, reproducible and requires only a small volume of semen (100 μ l) for each test which allows replicate testing in the same sample. The test depends on the ability of the spermatozoa to swim through 5 μ pores distributed throughout an ultrafilter membrane (Nucleopore). In the original TMMR technique (Hong *et al.*, 1981a), 100 μ l of the drug-semen mixture is placed in a cylinder whose base is formed by the membrane (upper chamber). This is immersed in a vial containing 2 ml phosphate buffer saline (PBS) (lower chamber). The vial is incubated for 2 h at 37° C. The pores in the membrane allow mixing between the two solutions on each side of the membrane and this would theoretically result in a 1:21 fold dilution of the concentration of the drug initially mixed with the semen, a factor

which has not been considered in the original method and which might affect the interpretation of the results of this technique. In this study the extent and consequences of this dilution are examined. Propranolol was chosen because its effect on sperm motility has previously been examined by the TMMR method (Hong *et al.*, 1981b).

Methods

To demonstrate the passage of propranolol through the membrane, 14 C radio-labelled propranolol was used. 100 μ l of 18 μ M [14 C]-propranolol solution (equivalent to 0.002 μ Ci radioactivity) was placed in the upper chamber. Sixteen chambers were prepared in this study. The radioactivity was measured in the lower chamber over a period of 30 min (1, 2.5, 5, 7.5, 10, 15 and 30 min incubation). Two chambers were analysed at each time point, and another two chambers were used as controls in which 100 μ l [14 C]-propranolol was mixed directly with 2 ml PBS. To examine the effect of dilution on

the TMMR we compared three variations of the method on normal semen samples from eight different healthy volunteers. Only semen samples with a sperm concentration higher than 15 million ml^{-1} and with more than 20% of progressive forward motility were used. A concentration of 0.8 mmol propranolol was used because it represents the concentration of propranolol which inhibited sperm motility by 50% (EC_{50}) in previous studies (Hong *et al.*, 1981b). The drug was put (i) in the upper chamber only (the original way of doing the test), (ii) the lower chamber only and (iii) in both the upper and lower chambers. Statistical analysis was done using two-way analysis of variance to compare the three groups and a Student's paired *t*-test and two tail test of significance for comparison between the individual groups.

Results

Figure 1 shows the increase in the concentration of propranolol in the lower chamber after placing the radio-labelled propranolol in the upper chamber. After 30 min the concentration in the lower chamber approximated very closely to that predicted after mixing without the presence of the membrane (0.78 vs $0.86 \mu\text{mol l}^{-1}$). The TMMR was expressed as a percentage of a control which was included in every experiment in which 100 μl semen is mixed with 50 μl PBS only. The results are shown in Figure 2. The percentage inhibition of sperm motility if the drug is placed in the upper chamber only is 51.7%, which is in agreement with the results found previously (Hong *et al.*, 1981b). There was a statistically significant difference between the three groups ($P < 0.001$). However there

Figure 1 Concentration of propranolol in the lower chamber after putting ^{14}C radio-labelled propranolol in the upper chamber.

Figure 2 The effect of propranolol on human sperm motility, expressed as % of control TMMR, using three different versions of TMMR technique (mean $\pm$ s.e. mean). * $P > 0.5$, ** $P < 0.001$.

was no statistically significant difference if propranolol was placed in the lower chamber only ($P > 0.5$), but there was a highly significant difference ($P < 0.001$) if the drug was placed in both upper and lower chambers from that if the drug was placed in the upper chamber only, leading to over-estimation of the EC_{50} in the original technique.

Discussion

In the original report of the TMMR test, propranolol mixed with semen in the upper chamber was diluted by the PBS in the lower chamber during incubation. It is known that the immobilising effect of propranolol on sperms will take place within 20 s (Chijioke *et al.*, 1986), a process which will take place even before putting the mixture on the membrane in the upper chamber. If this effect were irreversible, then dilution would have no effect on the TMMR. However, Peterson & Freund (1973) reported that the action of propranolol on spermatozoa cannot be reversed after prolonged periods of treatment (more than 20 min). Our results showed that within this 20 min period about 75% of the dilution had already taken place (Figure 1). Hong *et al.* (1981b) demonstrated that the effect of propranolol on sperm motility can be antagonised by caffeine, this reversibility occurring after treating spermatozoa with propranolol for 5 min (Hong, 1982). Our results indicate that reversal of the inhibition of sperm motility by propranolol can also occur after further dilution with PBS. The effect of dilution on other drugs tested by this method is unknown. Thus it cannot be concluded that the effect of propranolol shown in the original TMMR technique (method i) reflects the effect of its initial concentration

in the drug-semen mixture. To eliminate this source of error the PBS in the lower chamber should be replaced by a solution containing the same drug concentration in the upper chamber so that the concentration of drug on either side of the membrane is identical. This modification will ensure that there is no change in the drug concentration during the 2 h incubation. Using this modification the EC_{50} for the inhibitory effect of propranolol on sperm motility was found to be approximately 0.3 mmol l^{-1} (Gillan *et al.*,

personal communication). It is possible that the effects of other drugs on sperm motility previously determined by the TMMR method have been underestimated (Hong *et al.*, 1982, 1984, 1985; Jiang *et al.*, 1984; Louis & Pearson, 1985).

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