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The spermicidal and antitrichomonas activities of SSRI antidepressants☆

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Abstract—The study investigated spermicidal and antitrichomonas activities of selective serotonin reuptake inhibitor (SSRI) antidepressants with a view to generate new lead for development of dual-function spermicidal microbicides, which is an urgent global need. Fluoxetine, Sertraline, and Fluvoxamine exhibited both spermicidal and anti-STI (antitrichomonas) activities in vitro, whereas Paroxetine and Citalopram showed only the spermicidal activity. Fluoxetine exhibited better activity profile than the other antidepressant drugs with its spermicidal and antitrichomonas activities being comparable to that of the OTC contraceptive Nonoxynol-9. The non-detergent nature of Fluoxetine and a much lower spermicidal ED_{50} value (than N-9) may add considerably to its merit as a candidate for microbicidal contraceptive. Thus, the antidepressants exhibiting both spermicidal and antitrichomonas activities might provide useful lead for the development of novel, dual-function spermicidal contraceptives.

The worldwide increase in human immunodeficiency virus (HIV) and other sexually transmitted infections has made developing user-controlled, topical vaginal microbicides that provide protection against sexually transmitted disease (STD), an urgent global need.[1,2] Most heterosexual women want to reduce the risk of acquiring a STD[3] as well as to control their fertility. Extensive efforts[4–11] are being made to develop new agents with dual action, i.e., a spermicide with microbicidal activity that do not have the disadvantages of Nonoxynol-9 (N-9), the most widely used spermicide. These new agents must be safe, effective, acceptable, and affordable.[12] Human trials have shown that N-9 increases the risk of HIV transmission[13] and its breakdown products pose serious health and environmental risks.[14]

N-Ethylmaleimide, a sulfhydryl-selective alkylating agent,[15] and its derivatives have been found to possess spermicidal activities,[16] which is attributed to their interaction with the sulfhydryl groups present over sperm cell membrane.[17,18] Since binding to sulfhydryl groups of sperm membrane is important for the spermicidal activity, and paroxetine·HCl, a selective serotonin reuptake inhibitor (SSRI) antidepressant, is known to bind serotonin transporters by interacting with sulfhydryl groups,[15] it was suspected that SSRIs could possess spermicidal activity. Further, tricyclic antidepressants, Amytriptilin and Imipramine, have been shown to possess sperm immobilizing activity[19] and certain SSRI antidepressants, have been found to possess antifungal activity.[20] Additionally, serotonin functions as a neurotransmitter in brain, as well as in a number of other tissues including testis,[21] where it affects steroidogenesis by binding to cell surface receptors.

These observations prompted us to evaluate the spermicidal activity of certain currently used SSRI antidepressants, for example, Paroxetine, Fluoxetine, Sertraline, Citalopram, and Fluvoxamine (Fig. 1). Serotonin (Fig. 1) was also included in the study to compare its effect on sperm viability. Since spermatozoa and several of STD-causing microbes share common mechanisms of action,[22] it was considered worthwhile to test their spermicidal as well as anti-STI activity against *Trichomonas vaginalis*. N-9 was used as reference standard in this study.

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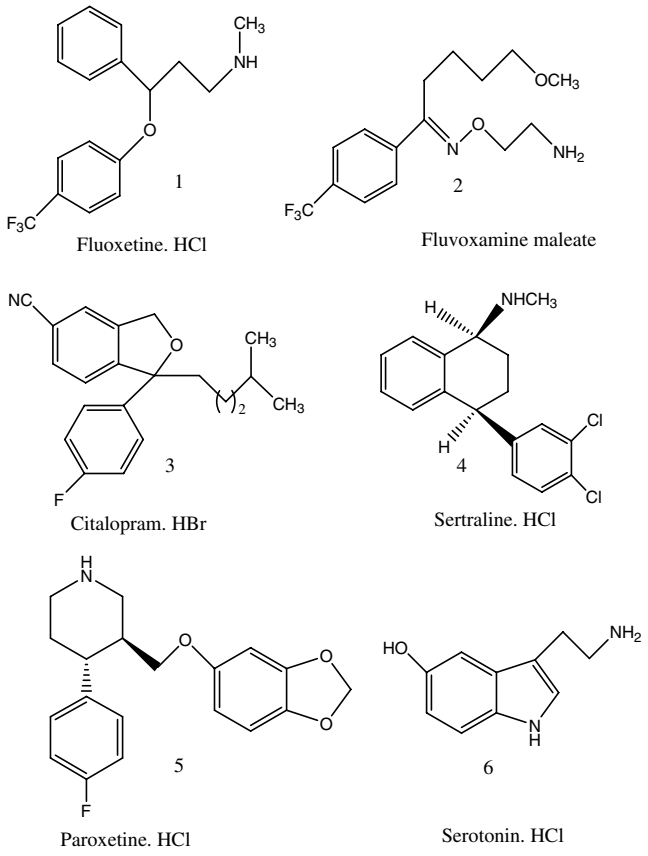

Figure 1.

Fluoxetine·HCl (1, Fig. 1) was synthesized by known procedure.[23] The other SSRIs (**2**–**5**) were extracted from the commercial tablets purchased from the local market according to the procedure given in British Pharmacopoeia.[24] These drugs (**1**–**5**) were characterized by spectral data. Serotonin·HCl (**6**) was procured from Sigma–Aldrich India Ltd.

Spermicidal minimum effective concentration (MEC) and ED_{50} were determined by modified Sander Cramer assay.[25,26] Briefly, the test compounds were dissolved in a minimum volume of physiological saline (0.85% sodium chloride in distilled water) to make a 1.0% (10 mg/ml) solution. The solutions were further diluted serially with saline. A spermicidal test was performed with each dilution starting from 1.0% until the minimum effective concentration (MEC) was arrived at. For this purpose, 0.05 ml of liquefied human semen was added to 0.25 ml of test solution and vortexed for 10 s. A drop of the mixture was placed on a microscope slide, covered with a cover glass and immediately examined under a phase contrast microscope. The results were scored positive if 100% spermatozoa became immotile in 20 s. The MEC was determined in three individual semen samples from different donors. For ED_{50} determination, the spermicidal solution was added to semen in the ratio of 5:1 and the percent motility of the spermatozoa was scored on a CASA (Computer Assisted Sperm Analyser; Model HTM-IVOS, Hamilton Thorn Research, USA). The motility at different concentrations of each test agent was recorded and ED_{50} value was determined from the motility versus the concentration curve. The institute's Ethical Committee approved all the experiments.

Trichomonas vaginalis parasites were grown in TYI-S-33 medium.[27] Parasites to be used in drug susceptibility assays were grown for one day following regular subculturing and were in the log phase of growth.

In vitro drug susceptibility assay was carried out using standard procedure.[28] All other test compounds leaving Citalopram·HBr were dissolved in phosphate-buffered saline (PBS). One milligram per millilitre stock solutions were prepared and serially diluted with PBS to obtain the required concentrations. Citalopram·HBr was not readily soluble in saline and was completely soluble only after overnight solubilization. However, this test compound was readily soluble in ethanol. Nonoxynol-9 dissolved in PBS was used as reference standard. The compounds were tested in the concentration range of 0.001–0.01%. 5×10^3 Trophozoites/well/ml were taken for the assay. Parasites were cultured anaerobically at 37 °C in the presence or absence of the test agent. Parallel culture containing ethanol (final concentration: 0.01%)/PBS served as control. Trophozoite growth was monitored on a daily basis by comparing the cultures containing the test agent with the corresponding vehicle control cultures. Antitrichomonas activity was assessed by Trypan blue staining to determine viability of the cells and cell number score and presented in terms of MLC and MLC_{50} after 48 h.

The results of spermicidal and antitrichomonas activities of the test agents are given in Table 1.

All the SSRI antidepressants evaluated in this study showed spermicidal activity with MEC ranging from 0.1 to 0.05% [Fluoxetine·HCl (MEC 0.05%), Paroxetine·HCl and Fluvoxamine maleate (MEC 0.1%) and Sertraline·HCl, and Citalopram·HBr (MEC 0.5%)]. Serotonin·HCl was inactive at 1% concentration. Pretreatment of spermatozoa with 1% Serotonin·HCl had no effect on spermicidal activity of Fluoxetine·HCl and Fluvoxamine maleate. The ED_{50} values for these SSRI antidepressants ranged from 0.00007% to 0.04% (Table 1).

Three antidepressants, Fluoxetine·HCl, Sertraline·HCl, and Fluvoxamine maleate, exhibited antitrichomonas activity with minimum lethal concentration (MLC) of 0.003%, 0.003%, and 0.005%, respectively. Their MLC_{50} ranged from 0.0012% to 0.003%. Citalopram·HBr and Paroxetine·HCl were inactive. The reference standard N-9 had MLC of 0.002% and MLC_{50} of 0.0009%.

The five SSRI antidepressants evaluated in this study displayed varying effect on viability of human sperm in vitro. Fluoxetine·HCl exhibited spermicidal MEC of 0.05%, which was equivalent to that of N-9,[29] the commercially available spermicide, while the other antidepressants were comparatively less active. Serotonin·HCl neither showed any spermicidal activity nor its pretreatment at a high concentration of 1% altered the spermicidal effect of Fluoxetine·HCl and

Table 1. Spermicidal and antitrichomonas activities of SSRI antidepressants

Antidepressant agent	Spermicidal activity		Antitrichomonas activity	
	ED_{50} (%)	MEC (%)	MLC_{50} (%)	MLC (%)
Fluoxetine·HCl	0.00007	0.05	0.0015	0.003
Citalopram·HBr	0.004	0.5	—	>0.01
Fluvoxamine maleate	0.005	0.1	0.003	0.005
Paroxetine·HCl	0.008	0.1	—	>0.01
Sertraline·HCl	0.04	0.5	0.0012	0.003
Serotonin·HCl	—	>1	—	—
Serotonin·HCl + Fluoxetine·HCl	0.00007	0.05	—	—
Serotonin·HCl + Fluvoxamine maleate	0.005	0.5	—	—
Nonoxynol-9	0.01	0.05	0.0009	0.002

Fluvoxamine maleate. This observation suggests that the spermicidal action of these SSRIs was not mediated via serotonin transporters. Spermicidal action of these SSRIs might be attributed to their effect on ATP synthesis by inhibition of oxidative phosphorylation in sperm mitochondria as exhibited by Fluoxetine·HCl in rat liver and brain mitochondria.[30] This inhibition by Fluoxetine·HCl was found to be non-specific and indirectly mediated via its interaction with phospholipids in the inner mitochondrial membrane.[30] The spermicidal activity of these SSRI antidepressants may also arise due to their possible interaction with sulfhydryl groups present over sperm membrane. This might be considered as their non-detergent mode of action.

Trichomonas vaginalis, the most common, non-viral STD, infects 250–350 million people worldwide every year causing serious discomfort to women with associated problems of adverse pregnancy outcome, pre-term delivery, low-birth-weight infants, infertility, and cervical cancer besides also an increase in the transmission of HIV.[31] The three SSRI antidepressants viz. Fluoxetine·HCl, Sertraline·HCl, and Fluvoxamine maleate have shown remarkable antitrichomonas activity comparable to that of N-9, the commercially available spermicide. This activity might be explained according to the finding that SSRIs primarily act at the serotonin transporter protein (SERT) and block the reuptake process of serotonin. SERT, with a molecular weight of 60–80 kDa and 12 transmembrane domains, is similar to other biogenic amine transporters.[20] It may be presumed that their antitrichomonas activity might be resulting from an interaction of SSRIs and membrane transport system, as has been reported for *Staphylococcus aureus* and chlorpromazine.[32]

It may be concluded from this study that the SSRI antidepressants exhibiting both the spermicidal and antitrichomonas activities may provide a lead structure for the development of novel, non-detergent, dual-function microbicidal spermicides. Of particular interest is fluoxetine with a noticeable spermicidal and microbicidal activity. Such molecules can be developed as suitable alternatives to N-9.

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