Potentiation of spermicidal activity of 2',4'-dichlorobenzamil by lidocaine

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The present investigation was designed to study the spermicidal activity of lidocaine, a membrane stabilizer, and its combination with 2',4'-dichlorobenzamil hydrochloride, a Na⁺-Ca²⁺ exchange inhibitor, on human semen and spermatozoa separated from semen. Both drugs per se produced dose- and time-dependent reduction in motility of ejaculated human sperm. Lidocaine was found to potentiate the spermicidal activity of benzamil resulting in significant decrease in time for producing complete loss of ejaculated sperm motility. Sperm revival test revealed irreversible loss of sperm viability indicating a spermicidal rather than spermistatic action by both the drugs. Furthermore, both benzamil (10-40 mM) per se and benzamil-lidocaine combination (0.5 and 16 mM) produced contraception in rabbit model.

A Na⁺-Ca²⁺ exchange inhibitor, 2',4'-dichlorobenzamil hydrochloride (DBZ), has been shown to produce irreversible loss of sperm viability in ejaculated human semen and spermatozoa separated from semen. DBZ is 1.62-fold more potent than widely used spermicide, nonoxynol-9. This action of DBZ has been found to be due to elevation of intrasperm calcium¹ and has been demonstrated to be potentiated by combining it with propranolol². Potentiation of action produced by combination of DBZ and propranolol resulted in significantly reduced time and dose of respective drugs for producing complete loss of ejaculated human sperm motility.

It is important to note that spermicidal activity of propranolol is due to its membrane stabilizing property and not due to β-blocking property³. However, propranolol has been reported to produce a fall in systolic blood pressure, heart rate and forced expiratory volume after insertion of vaginal tablets suggesting its systemic absorption from the vaginal mucosa⁴. This indicates that propranolol may not be safely used as a spermicide alone or in combination with DBZ and there is a need to study the potentiation of spermicidal activity of DBZ with another safe membrane stabilizer.

Lidocaine hydrochloride, a membrane stabilizer, has high water solubility and exhibits a pKa of 7.9 (Ref. 5). Hence, lidocaine hydrochloride is expected to be 1.2% unionized at pH 3.5-6.0 of vaginal fluid which is normally in the range of pH 3.5-6.0. Due to lower amount present in the lipid soluble (unionized) form, it is envisaged that systemic absorption of lidocaine across the vaginal mucosa would be very low.

Therefore, the present investigation aimed at studying the spermicidal activity of lidocaine hydrochloride and its combination with DBZ in samples containing ejaculated human semen and spermatozoa separated from semen. Furthermore, contraceptive efficacy of DBZ per se and that of its combination with lidocaine was studied in rabbit model.

Materials and Methods

2',4'-dichlorobenzamil hydrochloride, lidocaine hydrochloride and carbopol 934P were obtained as gift samples from SRI (USA), Astra-IDL (India) and Ranbaxy Research Laboratories (India), respectively. Quin-2AM was purchased from Sigma Chemicals (USA). All other chemicals were of AR grade and were purchased from S.D. Fine Chemicals Limited (India).

Semen collection and separation of spermatozoa—Semen was collected by masturbation from five non-drinkers and non-smoking male volunteers having a mean spermatozoal count of >20 x 10⁶ spermatozoa/ml (permissible level according to WHO manual) with >76% normal sperm morphology. An abstinence period of not less than 48 hr and not more than 5 days was allowed between two collection periods⁶. Care was taken to avoid cold shock to ejaculated sperm by collecting the sample in a warm sterilized beaker. Fresh samples were allowed to liquefy and subjected to further investigations at room temperature (35°C). For separation of spermatozoa, liquefied semen was diluted with an equal volume of Biggers-Whittingham (BWW) medium⁷ and centrifuged at 1000 rpm for 10 min. The supernatant was discarded.

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and the procedure was repeated. The pellet so obtained was finally resuspended in BWW medium so as to yield a final spermatozoal count of $> 20 \times 10^6$.

**Total sperm count**—Liquefied semen was diluted (1:20) with formalin-bicarbonate solution (sodium bicarbonate 5g; formalin 1 ml; distilled water q.s 1000 ml). Spermatozoa were counted microscopically (40x magnification) using a Neubauer chamber as per the procedure laid down in the World Health Organization Manual9.

**Sperm viability**—Eosin stains the dead spermatozoa, whereas the plasma membrane of viable sperm remains unstained9. Stock solution of DBZ and lidocaine hydrochloride were prepared in BWW medium. The drug solution was mixed in equal proportion with liquefied semen or spermatozoa separated from semen and incubated at 35°-37°C. In the studies employing combination of drugs, the solution of each drug was added to a sample of semen (0.5:0.5:1.0) and the mixture was incubated. Incubated samples were withdrawn at various time intervals, mixed with 0.5 ml eosin solution (0.5% w/v in normal saline) and observed microscopically. Fractional motility was calculated by the formula: % motile sperm in treated sample ÷ % motile sperm in control sample.

**Sperm revival test**—Glucose solution was added to the sample of immotile sperm so as to obtain a final concentration of 250mg/ml. The mixture was incubated at 35°-37°C for 60 min and then observed for revival of sperm motility9.

**Measurement of intracellular Ca$^{2+}$**—Effect of lidocaine and its combination with DBZ on intracellular Ca$^{2+}$ was studied in spermatozoa separated from human semen by using fluorescent dye, Quin-2AM (acetoxyethyl ester of Quin-2) according to the method outlined by White et al10.

**Contraceptive efficacy testing in rabbit model**—The drugs were incorporated in a carbopol gel formulation (carbopol 934P, 1.25; triethanolamine, 0.81; EDTA, 0.008; methyl paraben, 0.18; propyl paraben, 0.02; propylene glycol, 5.18; water 92.55 %w/v) for delivering them into the vagina of rabbits. Parabens and EDTA were dissolved in $1/4$th quantity of water by warming and allowed to cool. Carbopol was separately added to the remaining quantity of water in small increments with stirring. To this carbopol dispersion, DBZ (10, 20 or 40 mM) or a combination of DBZ (0.5 mM) and lidocaine hydrochloride (16 mM) was added. Adding triethanolamine (drop wise) completed gel formation. Finally, paraben solution and propylene glycol were added.

A cervicovaginal cannula was prepared using a syringe (5 ml) and a polyethylene tube (15 cm long, inner diam 2.75 mm; outer diam 3.25 mm) for delivering the gel into vagina of doe. A plastic bulb (0.75 inch long) having a hole in the center was attached to the free end of the polyethylene tube that served as a guide for the tube during its travel to the cervix-vagina.

The doe was held in left arm with its back flat and straight. The tip of the plastic bulb attached to polyethylene tube was slowly inserted into vaginal opening and gently pressed inside. Care was taken in maneuvering the bulb so that it does not enter the bladder by rupturing it. Beyond uro vaginal sphincter, the bulb was gently inserted up to the base of the cervix. Then the gel was passed through the cervicovaginal cannula by application of steady pressure on the syringe piston. After this the cannula was gently removed from the vagina and the doe was kept in supine position for 5 min before allowing it to return to the normal position.

After allowing 10 min for even distribution of gel in the doe vagina, the doe was placed in a cage along with a buck (known fertility) for mating. The doe was separated immediately after one mating and quarantined till 40 days for observation of delivery of litter. The doe used for this study had been quarantined for 40 days in order to ensure no carry over of pregnancy to the study period and were ensured for their being in the receptive phase after examination of red colour of their vulva11. Five doe were used for each group.

**Results and Discussion**

Both DBZ and lidocaine produced time- and dose-dependent reduction of sperm motility in ejaculated human sperm and spermatozoa separated from semen samples. Complete loss of sperm viability immediately on addition to ejaculated human semen was produced by DBZ at a concentration of 4 mM (Fig. 1). The same effect in samples containing spermatozoa separated from semen was obtained by adding DBZ at a concentration of 0.5 mM (Fig. 2). However, lidocaine required higher concentration of 28 mM in semen samples and 24 mM in samples containing spermatozoa separated from semen for immediately producing complete loss of sperm viability (Fig. 3). Eosin staining revealed dead sperm to be stained red after treatment with both DBZ and lidocaine. A combination of lidocaine (16 mM) and DBZ (0.5 mM) produced complete loss of sperm viability immediately after addition to semen samples suggesting potentiation.
of spermicidal activity of DBZ (Table 1). A negative result for sperm revival at the end of the experiments suggested spermicidal rather than spermistatic action by DBZ, lidocaine and their combination. Carbopol gel formulations containing DBZ (10-40 mM) and a combination of DBZ (0.5 mM) with lidocaine (16 mM) were found to be effective contraceptives in female rabbits (Table 2).

Results showed that DBZ was more potent spermicidal than lidocaine in both semen and spermatozoa separated from semen samples. However, the difference in spermicidal potency of lidocaine in presence or absence of seminal fluid was less pronounced than DBZ. Although no exact reason for the reduced potency of spermicidal agents in semen samples could be suggested, the role of higher viscosity and partial neutralization of the agents by constituents of seminal fluid cannot be ruled out.\(^6\,12\)

Reduction of sperm motility by DBZ has been reported to be accompanied with an elevation of intrasperm calcium\(^1\). Furthermore, propranolol that exhibits spermicidal activity due to its membrane stabilizing property has been shown to elevate intrasperm calcium\(^10\) and potentiate the spermicidal activity of DBZ\(^2\). It is noteworthy that neither lidocaine per se nor its combination with DBZ was found to elevate the intrasperm Ca\(^{2+}\) in the present study.

However, lidocaine was found to potentiate the spermicidal activity of DBZ. DBZ per se produced complete loss of sperm viability in 550, 300, 230 and 110 min at a concentration of 0.25, 0.5, 1.0 and 2.0 mM, respectively. Lidocaine per se (16 mM) produced complete loss of sperm viability in semen and spermatozoa separated from semen at 50 and 40 min, respectively. This concentration of lidocaine was chosen for potentiation studies so that the reduction in spermicidal time in combination with DBZ could be more apparent. It is evident from Table 1 that lidocaine (16 mM) produced complete loss of sperm viability in semen samples at 10, 1, 1 and 1 min, when combined with 0.25, 0.5, 1.0 and 2.0 mM concentrations of DBZ, respectively. This indicated a reduction...
Table 1—Fractional motility of sperm in (A) human semen and (B) spermatozoa separated from semen following combined treatment of lidocaine (16 mM) and different concentrations (mM) of 2',4'-dichlorobenzamid hydrochloride (DBZ)*

| Time (min) | *0.1 | *0.25 | *0.3 | *0.4 | *0.5 | *1.0 | *2.0 | *0.1 | *0.2 | *0.3 | *0.4 | *0.5 | *1.0 | *2.0 |
|-----------|-----|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 1         | 0.45 ± 0.023 | 0.48 ± 0.026 | 0.54 ± 0.033 | 0.28 ± 0.031 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 5         | 0.24 ± 0.025 | 0.15 ± 0.019 | 0.03 ± 0.017 | 0.03 ± 0.017 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 10        | 0.05 ± 0.017 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 15        | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     |

Blank rows indicate that the experiment was terminated after observing 100% immotile sperm.

![Graph showing fractional motility](image)

Fig. 3—Influence of various doses of lidocaine on motility of sperm in ejaculated human semen (solid lines) and spermatozoa separated from semen (broken lines) samples.

Table 2—Contraceptive efficacy testing in female rabbits

<table>
<thead>
<tr>
<th>Control</th>
<th>DBZ conc. (mM)</th>
<th>40</th>
<th>20</th>
<th>10</th>
<th>0.5 + LDN (16 mM)</th>
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<tbody>
<tr>
<td>DBZ</td>
<td></td>
<td>+</td>
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<tr>
<td>0.5</td>
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<tr>
<td>LDN</td>
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<tr>
<td>0.5 + LDN</td>
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Note: (+) indicates conception in doe; (*) indicates contraception in doe.

... of time for producing complete loss of sperm viability in semen samples by 300-fold by using a combination of lidocaine and DBZ. Similarly, the time for producing complete loss of sperm viability of spermatoza separated from semen decreased from 60, 30 and 10 min (DBZ per se) to 10, 5 and 1 min by combining lidocaine (16 mM) with DBZ at concentrations of 0.1, 0.2 and 0.5 mM, respectively.

Although the data generated in the present study could not suggest the exact mechanism of potentiation, the results nevertheless indicated a highly significant potentiation of spermicidal activity of DBZ in combination with lidocaine in ejaculated human semen and contraceptive activity in female rabbits. This strategy will help in reducing the dose of respective drugs in a contraceptive formulation.

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References


