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Short communication

Effect of 2′,4′-dichlorobenzamil hydrochloride, a Na^+–Ca^{2+} exchange inhibitor, on human spermatozoa

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Abstract

The present study is aimed to investigate the contact spermicidal efficacy of 2′,4′-dichlorobenzamil hydrochloride (DBZ), a Na^+–Ca^{2+} exchange inhibitor, on ejaculated human spermatozoa. The drug produced a dose- and time-dependent spermicidal action on human spermatozoa. A concentration of 4 mM produced total loss of sperm viability within 1 min of addition to total semen. On the other hand, a similar action on spermatozoa separated from semen was noted at 0.5 mM concentration. The loss of spermatozoal viability was accompanied with an increase in intracellular Ca^{2+}. Sperm revival testing with glucose suggested a spermicidal rather than a spermiostatic action.

Keywords: 2′,4′-Dichlorobenzamil; Na^+–Ca^{2+} exchange inhibitor; Spermicide, contact

1. Introduction

An increase in cytosolic Ca^{2+} is reported to be detrimental for sperm motility and viability. The existence of a Na^+–Ca^{2+} exchanger has been demonstrated on sperm membrane (Babcock and Pfeiffer, 1987). Amiloride and its derivatives that inhibit Na^+–H^+ and Na^+–Ca^{2+} exchange are reported to increase intracellular levels of Ca^{2+} in spermatozoa (Breitbart et al., 1990). Moreover, the spermicidal action of propranolol is accompanied by an intracellular increase of Ca^{2+} in spermatozoa (White et al., 1995). 2′,4′-Dicholorobenzamil is an amiloride derivative and is a selective inhibitor of Na^+–Ca^{2+} exchange (Deshpande et al., 1997). In light of the reports mentioned above, the present study was designed to investigate the effect of 2′,4′-dichlorobenzamil hydrochloride on ejaculated human spermatozoa.

2. Materials and methods

2′,4′-Dichlorobenzamil hydrochloride (DBZ) was a gift from Research Biochemical (USA). Eosin (Y) dye was purchased from E. Merck (India). Bovine serum albumin was purchased from CDH (India). All other chemicals (AR grade) were purchased from SD Fine Chemicals (India).

2.1. 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 $> 20 \times 10^6$ spermatozoa/ml with $> 76\%$ normal sperm morphology. An abstinence period of not less than 48 h and not more than 5 days was allowed (Reynolds and Narang, 1984). The fresh samples were allowed to liquefy and further investigations were carried out at room temperature (35°C). For separation of spermatozoa, liquefied semen was diluted with an equal volume of Briggers Whitten and Wittingham (BWW) medium (NaCl 95 mM; KCl 4.8 mM; $CaCl_2$ 1.3 mM; KH_2PO_4 1.2 mM; $MgSO_4 \cdot 7H_2O$ 2.4 mM; $NaHCO_3$ 25 mM; sodium lactate 1 mM; sodium pyruvate 1.2×10^{-4} mM; glucose 5.5 mM; bovine serum albumin 3.3%) and was centrifuged at 1000 rpm for 10 min. The supernatant was discarded and the procedure was repeated. The pellet so obtained was finally resuspended in BWW medium so as to yield a final spermatozoal count of $> 20 \times 10^6$.

2.2. Total sperm count

Liquefied semen was diluted (1:20) with formalin–bicarbonate solution (sodium bicarbonate 5 g; formalin 1.0

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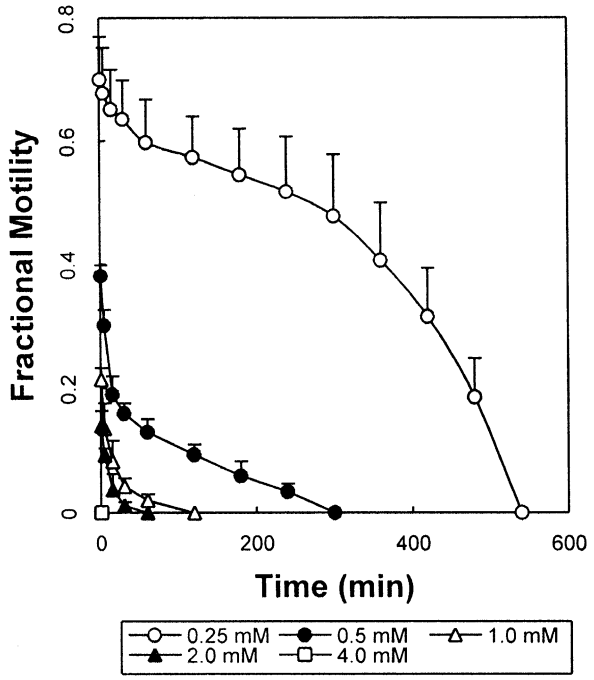

Fig. 1. Dose- and time-dependent influence of DBZ on human spermatozoa in total semen. Semen was mixed with various concentrations of DBZ solution and incubated at 35–37°C. Samples were observed microscopically at different time intervals as described in Section 2. Fractional motility was calculated as the ratio of % motile sperm in presence of drug to the % motile sperm in the control sample. The values represent the means ± S.D. for five volunteers.

ml; distilled water qs 1000 ml). Spermatozoa were counted microscopically at 40 × magnification using a Neubauer chamber as per the procedure laid down in the World Health Organization Manual (WHO Manual, 1999).

2.3. Sperm viability

Eosin stains the dead spermatozoa whereas the plasma membrane of viable sperm is impermeable to this dye (WHO Manual, 1999). A stock solution of dichlorobenzamil hydrochloride was prepared in BWW medium and mixed in equal proportion with liquefied semen or spermatozoa and incubated at 35–37°C. The effect of DBZ on sperm viability was evaluated at 0.25, 0.5, 1.0, 2.0 and 4.0 mM concentration on total semen and at 0.05, 0.1, 0.25 and 0.5 mM concentration on spermatozoa. Incubated samples were mixed with 0.05 ml eosin solution (0.5% w/v in normal saline) and observed microscopically after 1, 5, 15, 30, 60 min and thereafter after every 60 min of drug treatment.

2.4. Sperm revival test

A glucose solution was added to the sample of immotile spermatozoa so as to obtain a final concentration of 250 mg/ml. The mixture was incubated at 35–37°C for 60 min and then observed for revival of sperm motility (Reddy et al., 1996).

2.5. Measurement of intracellular Ca^{2+}

The effect of DBZ on intracellular Ca^{2+} was studied in spermatozoa separated from semen by using the fluorescent dye, Quin 2-AM (acetoxymethyl ester of Quin-2) according to the method outlined by White et al. (1995).

3. Results

DBZ produced a dose- and time-dependent decrease in sperm motility after addition to a liquefied semen sample (Fig. 1). Total loss of sperm viability within 1 min of addition to total semen was noted at 4 mM concentration. On the other hand, complete loss of viability of spermatozoa separated from semen was observed at 120, 60 and 15 min with 0.05, 0.1 and 0.25 mM DBZ, respectively. A concentration of 0.5 mM of DBZ was enough to produce total loss of viability within 1 min of addition to spermatozoa separated from semen. The decreased viability of spermatozoa was accompanied with an increase in the

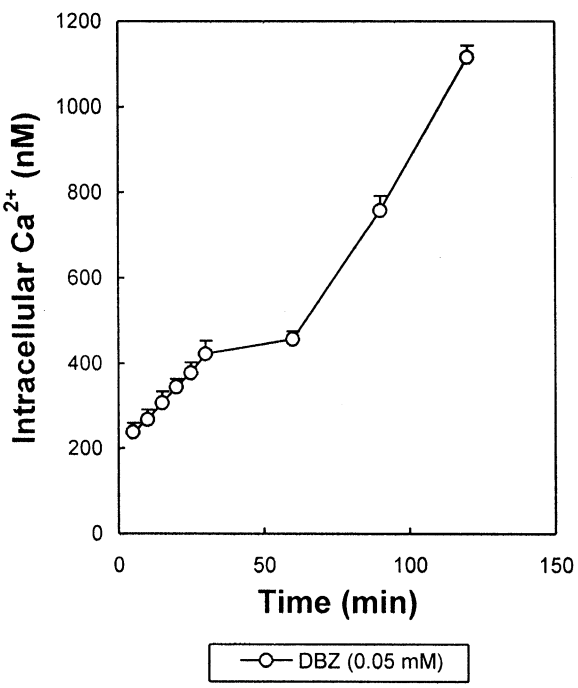

Fig. 2. Influence of DBZ (0.05 mM) on intracellular Ca^{2+} concentration in spermatozoa. A solution of Quin 2-AM (50 μM) was added to a suspension of spermatozoa separated from semen and was incubated at 37°C for 20 min. After dilution with fresh BWW medium and incubation at 37°C for a further 90 min, the suspension was washed thrice with BWW medium to remove extracellular Quin 2-AM. The pellet was finally suspended in BWW medium and the drug solution was added. Fluorescence was measured at various time intervals at excitation and emission wavelengths of 339 and 492 nm, respectively.

intracellular Ca^{2+} level from 200 ± 51 nM (basal) to 1100 ± 26 nM (final) in the presence of either concentration of DBZ (0.05, 0.1, 0.25 or 0.5 mM). The increase in intracellular Ca^{2+} in the presence of 0.05 mM concentration of DBZ is shown in Fig. 2. The lack of revival of sperm motility showed that DBZ had a spermicidal rather than spermiostatic action in both semen and spermatozoa separated from semen samples.

4. Discussion

DBZ produced a dose- and time-dependent loss of sperm motility in both semen (Fig. 1) and spermatozoal samples. It is worth noting that the spermicidal efficacy of DBZ was increased eightfold in spermatozoa separated from semen. This tentatively suggests that semen may contain some substances that can bind to DBZ and may decrease its access to the sperm membrane. Alternatively, a decreased viscosity of spermatozoa may lead to an increased rate of diffusion of DBZ to its site of action on the sperm membrane. Our results are supported by a similar observation with magainins which are also more potent contact spermicides on spermatozoa than on total semen (Edelstein et al., 1991; Reddy et al., 1996).

The intracellular Ca^{2+} level of spermatozoa increased approximately sixfold within 120 min of the addition of 0.05 mM DBZ (Fig. 2). This was accompanied by a decrease in spermatozoal motility, strongly indicating the role of elevated intracellular Ca^{2+} in producing a spermicidal action.

It is noteworthy that nonoxynol-9 produces complete loss of viability of spermatozoa at 0.81 mM concentration (White et al., 1995). However, DBZ produced the same effect at 0.5 mM concentration. Hence, the spermicidal efficacy of DBZ is 1.62 times more than that of nonoxynol-9.

On the basis of the results, it may be suggested that DBZ, a Na^{+}–Ca^{2+} exchange inhibitor, produces a potent spermicidal action on human spermatozoa by elevating the intracellular Ca^{2+} and has a potential to be developed as a contact spermicide.

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