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Spermicidal activity of sulfonylureas and meglitinide analogues: role of intrasperm Ca^{2+} elevation

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Abstract

Intrasperm calcium concentration ($[Ca^{2+}]_{is}$) is known to play a vital role in regulating motility and viability of ejaculated spermatozoa. K_{ATP} channel blockers are reported to block K_{ATP} channels, leading to depolarization of the cell membrane. This activates the voltage-gated calcium channels, resulting in enhanced Ca^{2+} influx, which eventually elevates the intracellular $[Ca^{2+}]$ level. Hence, it can be hypothesized that drugs acting on K_{ATP} channels could possess the ability to elevate $[Ca^{2+}]_{is}$. Sulfonylureas such as glibenclamide or gliclazide, as well as meglitinide analogues such as repaglinide, produced a dose- and time-dependent decrease in viability, each requiring 7.5 mM, 10 mM and 6.5 mM, respectively, to produce death of all sperm cells immediately upon addition to ejaculated human semen samples. The reduction in sperm viability was accompanied by an elevation of $[Ca^{2+}]_{is}$ and was affected by removal of extracellular Ca^{2+}. Significantly ($P < 0.05$) less time was required to elevate $[Ca^{2+}]_{is}$ and produce complete loss of sperm viability when any of these drugs were added to sperm cells simultaneously with selected agents affecting Ca^{2+} homeostasis. Thus, the spermicidal activity of these drugs attributed to elevation of $[Ca^{2+}]_{is}$ and their synergism can be potentially exploited for developing contact spermicidal formulations.

Introduction

In ejaculated spermatozoa, $[Ca^{2+}]_{is}$ regulates various sperm functions such as motility and hyperactivation (Carlson et al 2003), chemotaxis (Spehr et al 2003) and acrosome reaction (Kirkman-Brown et al 2002). Capacitation, a decrease in extracellular calcium below 60% or an increase in $[Ca^{2+}]_{is}$ above 1200 nM (Gupta et al 2005) leads to death of ejaculated spermatozoa. Spermatozoa possess sophisticated mechanisms for regulation of $[Ca^{2+}]_{is}$ through influx and efflux of Ca^{2+} via various ion channels and pumps present on sperm membrane. Hence, these channels can be targeted for modulating $[Ca^{2+}]_{is}$ and the pharmacological agents acting on these channels can be exploited as contact spermicides. Pharmacological modulation of $[Ca^{2+}]_{is}$ has been investigated by treating human ejaculated semen samples with propranolol (White et al 1995), chelating agents (Lee et al 1996), 2′, 4′-dichlorobenzamil hydrochloride (Patni et al 2001; Reddy et al 2001), membrane stabilizers (Moudgil et al 2002a, b), H_2-receptor antagonists (Gupta et al 2003), H_1-receptor antagonists (Gupta et al 2004), IP_3 modulators and Ca^{2+}-ATPase inhibitors (Gulati et al 2006). These studies revealed that an elevation of $[Ca^{2+}]_{is}$ to ~1200 nM produced complete loss of viability of human ejaculated sperm cells. Pharmacological modulation for achieving this critical $[Ca^{2+}]_{is}$ threshold seems to offer a feasible approach for contact spermicidal activity (Gupta et al 2005).

Antidiabetic drugs belonging to the class of sulfonylureas or meglitinide analogues are reported to possess the ability to block K_{ATP} channels. The blockade of K_{ATP} channels causes membrane depolarization, which leads to opening of voltage-operated calcium channels. As a consequence, there is enhanced Ca^{2+} influx as well as insulin secretion in pancreatic β-cells (Ashcroft 2005).

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Glibenclamide (GBE) is reported to block K_{ATP} channels in pancreatic β-cells (Ashcroft et al 1999) and increases calcium content in cultured human peripheral blood mono nuclear cells (Alon et al 2006). Gliclazide (GLE) is reported to block K_{ATP} channels in mouse isolated pancreatic β-cells (Lawrence et al 2001) and attenuates the intracellular Ca^{2+} changes induced in-vitro by ischaemia in the retinal slices of rats with streptozotocin-induced diabetes (Kinukawa et al 2005). Furthermore, meglitinide analogues such as repaglinide (RGE) and GLE are reported to elevate the cytosolic Ca^{2+} concentration in βTC3 cells and in pancreatic β-cells of rat due to blockade of K_{ATP} channels (Gromada et al 1995). In addition, RGE blocks K_{ATP} channels in cloned β-cells and cardiac and smooth muscle cells (Dabrowski et al 2001).

It has been recently demonstrated by employing immunocytochemistry that K_{ATP} channel subunits are present in adult mouse sperm. The K_{ir} 6.1 subunit was detected in the flagellum midpiece, while the K_{ir} 6.2 subunit was localized in the flagellum midpiece as well as in the post-acrosomal region of the sperm head. The sulfonylurea receptor-1 subunit displays the same distribution pattern as K_{ir} 6.2 in the flagellum midpiece and in the post acrosomal region of the sperm head. The sulfonylurea receptor-2 subunit was strongly immunolabelled in the flagellum principal piece, and to a lesser extent in the flagellum midpiece (Acevedo et al 2006). In the light of these reports it can be hypothesized that drugs belonging to the class of sulfonylureas as well as meglitinide analogues may prove useful in modulating $[Ca^{2+}]_{is}$, similar to their effect in pancreatic β-cells, βTC3 cells, retinal slices and human peripheral blood mononuclear cells.

The use of $[Ca^{2+}]_{is}$ elevation to arrest the viability of human ejaculated spermatozoa is expected to be much safer than the use of commonly available spermicides because the latter are mostly surfactant in nature (nonoxynol-9 and cetrimide) and their frequent use is associated with altered vaginal cell morphology and permeability (Stafford et al 1998). Thus, the present study aimed at evaluating the influence of GBE, GLE and RGE on sperm motility and viability. In addition, $[Ca^{2+}]_{is}$ modulation by these drugs and other agents capable of influencing Ca^{2+} homeostasis was studied in an attempt to correlate loss of sperm viability with elevated $[Ca^{2+}]_{is}$ in human ejaculated semen samples.

Materials and Methods

GBE was a gift sample from Ind Swift Labs Ltd, Parwanoo, India. GLE was a gift sample from Panacea Biotech, Lalru, India. RGE was a gift sample from Torrent Pharmaceutical Ltd, Gandhinagar, India. Miconazole (MZE) was a gift sample from Micro Labs Ltd, Tamil Nadu, India. 2′; 4′-Dichlorobenzamil hydrochloride (DBZ) was a gift from SRI, Menlo Park, CA, USA. Diazoxide (DXE) and Quin 2-AM were purchased from Sigma Chemicals, USA. All other chemicals were of analytical reagent grade.

Preparation of drug solutions

A solution containing 2% v/v dimethylsulfoxide (DMSO) in Biggers–Whitten–Whittingham (BWW) medium was used for preparing stock solution of GBE, GLE, RGE or DXE. Further dilutions of these solutions were made with BWW medium. Solution of DBZ was prepared in BWW medium. MZE was dissolved in a solution containing absolute ethanol 1% (v/v) in BWW medium. Quin2-AM was dissolved in DMSO. Ca^{2+}-free BWW medium was prepared by substituting calcium chloride with an equimolar concentration of sodium chloride in the normal BWW medium together with the addition of EDTA (100 μM).

Semen collection

Semen samples exhibiting $> 20 \times 10^6$ spermatozoa mL^{-1}, $> 60\%$ motility and $> 60\%$ normal morphology (World Health Organization 1999) were collected from human volunteers (22–25 years old) by masturbation into a warm, sterile glass beaker. Freshly ejaculated samples from six volunteers after an abstinence from sexual activity of not less than 48 h but not more than 5 days were used for all experiments. Each experiment was carried out on the semen sample of each volunteer (crossover design). All volunteers were instructed not to smoke or consume liquor during the study period.

The protocol was approved by the Institutional Ethical Committee of the Department of Pharmaceutical Sciences and Drug Research, Punjabi University, Patiala, India. All volunteers were informed and written consent was obtained prior to their enrolment in this study.

Sperm motility analysis

The effect of GBE, GLE, RGE, DBZ or MZE alone on sperm motility was studied by mixing their respective solutions with an equal volume of liquefied semen.

Experiments aimed at studying the effect of combinations of DBZ and MZE with GBE, GLE or RGE on sperm motility employed the addition of the two drug solutions and semen in the ratio of 0.5:0.5:1.0. Experiments aimed at confirming K_{ATP} channel blockade and subsequent increase in intracellular calcium to be the main mechanism involved in spermicidal action of these agents comprised of sperm motility and $[Ca^{2+}]_{is}$ measurements after addition of DXE to drug solutions and liquefied semen sample in the ratio of 0.5:0.5:1.0.

The drug–semen admixtures were incubated at 37 ± 2°C. A sample (0.1 mL) was removed at different time intervals, gently mixed with 0.05 mL eosin Y dye solution (0.5% w/v in normal saline), and examined for dead (stained red) and alive (unstained) spermatozoa. Not less than 200 sperm cells were counted and the results expressed as fractional motility. Fractional motility was calculated by the following formula: fractional motility = % motile sperm in drug-treated semen sample/% motile sperm in control semen sample.

Sperm revival test

Glucose solution was added to a sample of totally immotile sperm (final concentration of glucose adjusted to 250 mg mL^{-1}) and the mixture incubated for 60 min at 37 ± 2°C (Reddy et al 1996). After incubation, the specimen was examined for revival of sperm motility.

Measurement of $[Ca^{2+}]_{is}$

$[Ca^{2+}]_{is}$ was monitored in various drug–semen admixtures employed for sperm motility analysis. $[Ca^{2+}]_{is}$ was determined by measuring the fluorescence signal emitted by the Ca^{2+} chelating agent, Quin 2-AM, using a spectrofluorimeter (SL-174, Elico, India). The excitation and emission wavelengths employed were 339 nm and 492 nm, respectively. $[Ca^{2+}]_{is}$ was calculated according to the method reported by White et al (1995).

Data analysis

The results are expressed as mean ± s.d. of experiments conducted on semen samples from six volunteers. Analysis of variance followed by the Studentized range test was used for comparing the effect of selected doses of GBE, GLE or RGE with that of their respective combination with DBZ or MZE, as well as the effect of DBZ or MZE alone. This was done for data pertaining to both fractional motility as well as $[Ca^{2+}]_{is}$. The level of significance was fixed at $P < 0.05$. Statistica software 7.0 (StaSoft Inc., Tulsa, OK, USA) was used for these calculations.

Results and Discussion

Effect of GBE, GLE or RGE on sperm motility

GBE, GLE or RGE (Figure 1) produced a dose- and time-dependent decrease in sperm motility. A dose of 7.5 mM of GBE, 10 mM of GLE, or 6.5 mM of RGE was observed to produce complete loss of sperm viability immediately upon addition to ejaculated human semen samples. The dead sperm cells could not be revived even after incubation in glucose solution. This indicated irreversible loss of sperm cell viability, suggesting spermicidal activity of all these drugs. Further, removal of extracellular Ca^{2+} significantly ($P < 0.05$) delayed the time taken by selected concentrations of these drugs to produce complete loss of sperm motility. Hence, Ca^{2+} influx from an extracellular source appeared to play a major role in the spermicidal action of these drugs.

It is important to note that the concentration of GBE, GLE or RGE found to produce complete loss of sperm viability immediately upon addition to ejaculated human semen samples was much higher than that reported for RGE or GBE to induce a time-dependent increase in intracellular calcium in β-TC3 cells (Gromada et al 1995). This difference in response to added drugs could be attributed to a reduction of intimate contact of spermatozoa with drug molecules due to the viscosity of the semen. Also, the role of various constituents in semen that are reported to bind with added

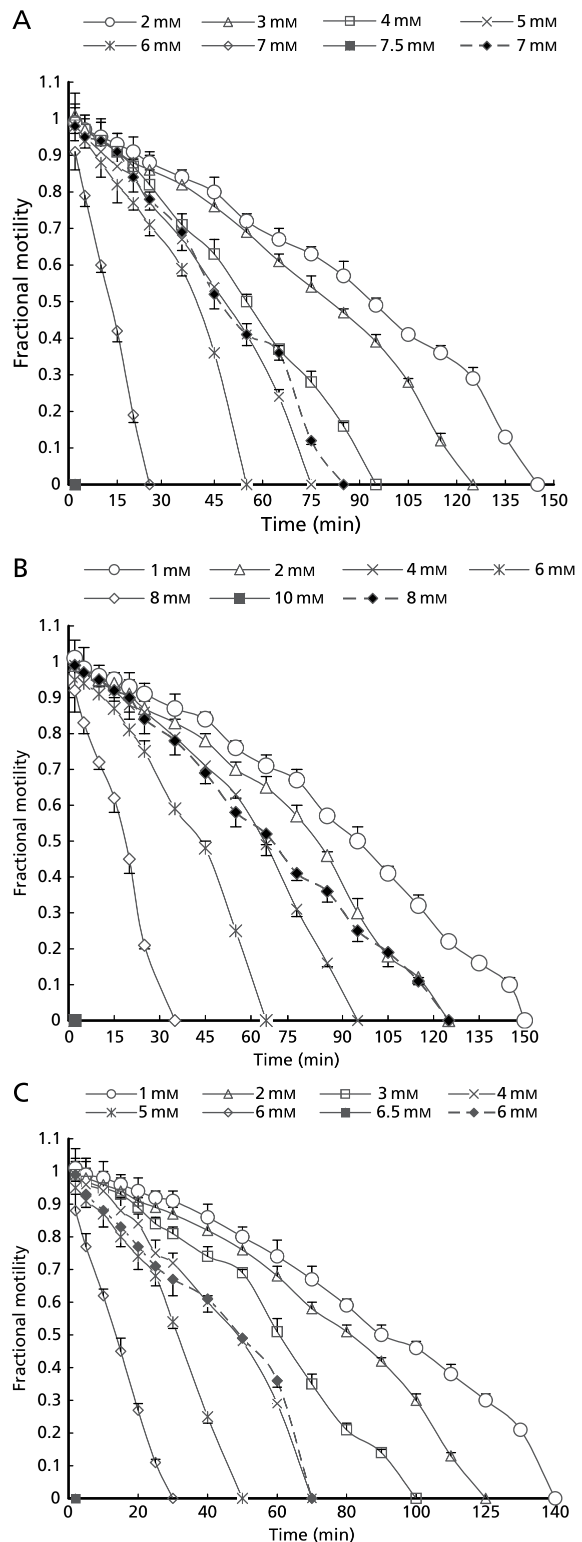

Figure 1 A. Effect of glibenclamide on ejaculated human sperm motility in the presence (solid lines) and absence (broken line) of extracellular calcium. B. Effect of gliclazide on ejaculated human sperm motility in the presence (solid lines) and absence (broken line) of extracellular calcium. C. Effect of repaglinide on ejaculated human sperm motility in the presence (solid lines) and absence (broken line) of extracellular calcium.

drugs and decrease their sensitivity towards spermatozoa cannot be ruled out. Other studies have also reported a higher concentration of magainins (Edelstein et al 1991; Reddy et al 1996) and 2′, 4′-dichlorobenzamil (Patni et al 2001; Reddy et al 2001) required to produce total loss of sperm viability in the presence of human semen as compared with that in spermatozoa separated from semen. Furthermore, the number of K_{ATP} receptors expressed in sperm cells vis-à-vis pancreatic cells could have played an important role in influencing the activity of these drugs in human ejaculated sperm cells. However, at this point it is not possible to suggest an exact reason for justifying the contention due to the limited data available.

Effect of GBE, GLE or RGE on $[Ca^{2+}]_{is}$

It is evident from Figure 2 that $[Ca^{2+}]_{is}$ remained constant after 25–40 min of treatment of semen samples with doses of drugs that had produced complete loss of sperm viability in approximately the same time in normal BWW medium. The same effect was observed in Ca^{2+}-free BWW medium upon treatment of semen samples with doses of drugs that had produced complete loss of sperm viability in 55–65 min. Therefore, the spermicidal action of these drugs could be ascribed to elevation of $[Ca^{2+}]_{is}$ to a threshold level of 1100–1200 nM. This finding was in agreement with earlier reports on ejaculated human sperm (White et al 1995; Lee et al 1996; Patni et al 2001; Reddy et al 2001; Gupta et al 2003, 2004; Gulati et al 2006).

Intracellular calcium in cultured human peripheral blood mononuclear cells is reported to increase after exposure to sulfonylurea drugs and this is a prerequisite for insulin release (Alon et al 2006). It is also reported that RGE and GBE (100 nM) induced a time-dependent increase in intracellular calcium in β-TC3 cells. The resting intracellular calcium in unstimulated β-TC3 cells amounted to $189 \pm 7\,nmol\,L^{-1}$ (n = 59). The calcium responses of 800–900 nM in amplitude were sustained and had a latency of 30–60 s. These results were shown in the presence of extracellular calcium. However, in the absence of extracellular calcium (addition of 100 μM EGTA), RGE did not evoke any increase in intracellular calcium, but subsequent change to calcium-containing medium caused an immediate increase of intracellular calcium. Furthermore, the elevation of intracellular calcium in pancreatic β-TC3 cells and rat pancreatic β-cells by RGE was completely inhibited by the addition of 10 μM verapamil, an L-type calcium-channel blocker. This confirmed that Ca^{2+} influx from an extracellular source was effective in elevating the intracellular calcium by these drugs. Further, the time course of change in intracellular calcium was strongly dependent on the applied drug concentration. With RGE the $\Delta([Ca^{2+}]_i)_{max}$ amounted to 731 ± 15 nM, with a half maximal effective concentration of 0.53 ± 0.04 nM, and with GBE it was found to be 715 ± 15 nM, with a half maximal effective concentration of 0.52 ± 0.04 nM. The increase in intracellular calcium by these drugs was ascribed to inhibition of K_{ATP} channels, thereby causing Ca^{2+} influx through voltage-dependent L-type calcium channels (Gromada et al 1995). GLE is reported to attenuate the intracellular Ca^{2+} changes induced in-vitro by ischaemia in the retinal slices of rats with streptozotocin-induced diabetes (Kinukawa et al 2005). It is evident from Figure 1 that elevation of $[Ca^{2+}]_{is}$ by GBE (7 mM), GLE (8 mM) or RGE (6 mM) in normal BWW medium was significantly ($P < 0.05$) faster as compared with that in Ca^{2+}-free BWW medium. This was manifested in significantly ($P < 0.05$) faster loss of motility in the presence of extracellular calcium as compared with that in the absence of extracellular calcium (Figure 1). Hence, the findings strongly suggested the role of Ca^{2+} influx in the spermicidal activity of these drugs.

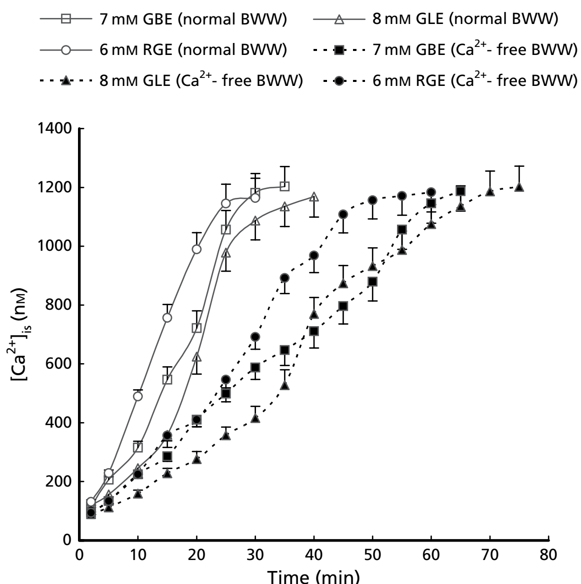

Figure 2 $[Ca^{2+}]_{is}$ levels (nM) after treatment with glibenclamide (GBE; 7 mM), gliclazide (GLE; 8 mM) or repaglinide (RGE; 6 mM) in ejaculated human spermatozoa. Solid and broken lines indicate incubation of sperm cells in normal or Ca^{2+}-free BWW medium.

Confirmation of involvement of K_{ATP} channels in spermicidal activity

The use of DXE (4 mM) along with GBE (7 mM), GLE (8 mM) or RGE (6 mM) in the presence of extracellular calcium prolonged the time required to produce complete loss of sperm motility by approximately 2-fold. The observed prolongation of the time required for spermicidal action in the presence of DXE was accompanied by a slow increase of $[Ca^{2+}]_{is}$ as compared with the effect of GBE, GLE or RGE alone in normal BWW medium (Figure 3). DXE, a K_{ATP} channel opener, is reported to repolarize the membrane, deactivate the voltage-gated calcium channels and, hence, decrease the Ca^{2+} influx (Dunne et al 1990). Therefore, the prolongation of time required for the spermicidal action of GBE, GLE or RGE could be attributed to the blockade of their K_{ATP} channel depolarizing activity by DXE. This finding confirmed that the spermicidal activity of the above drugs was due to inhibition of K_{ATP} channels present in sperm cells.

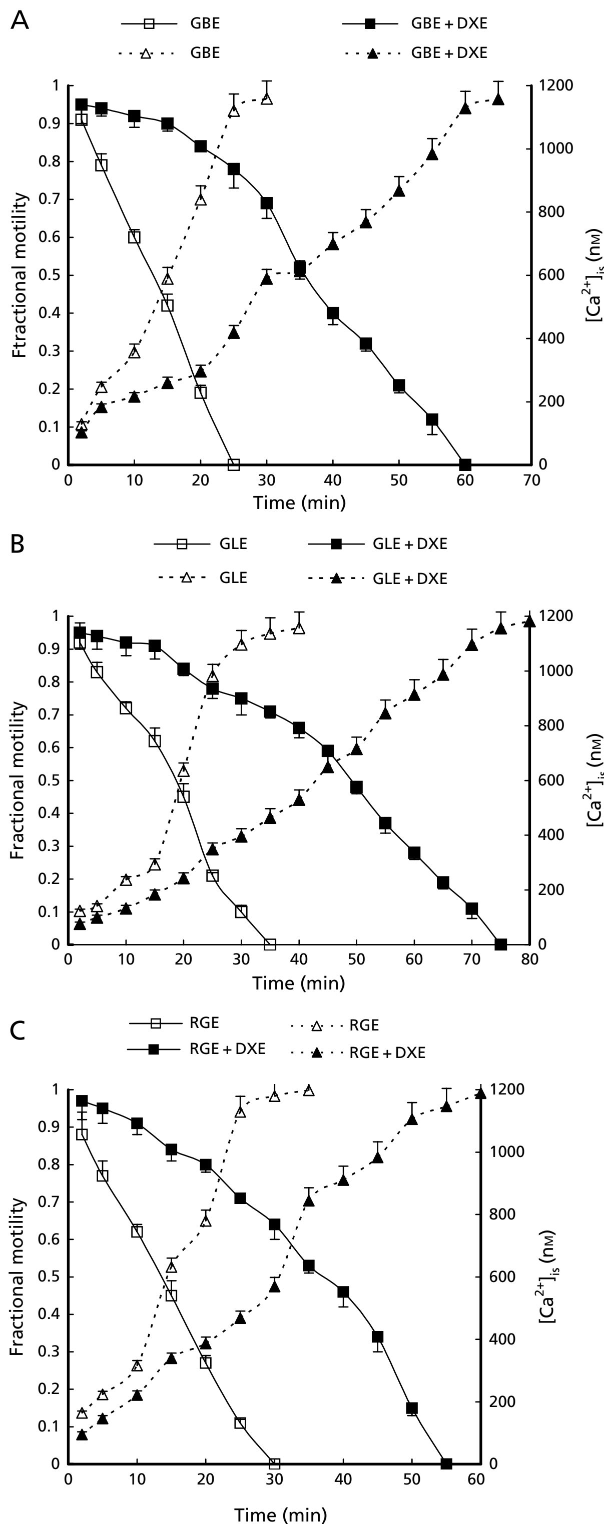

Figure 3 A. Influence of admixtures of diazoxide (DXE) (4 mM) and glibenclamide (7 mM) on motility (solid lines) and $[Ca^{2+}]_{is}$ (broken lines) of human ejaculated sperm in the presence of extracellular Ca^{2+}. B. Influence of admixtures of DXE (4 mM) and gliclazide (8 mM) on motility (solid lines) and $[Ca^{2+}]_{is}$ (broken lines) of human ejaculated sperm in the presence of extracellular Ca^{2+}. C. Influence of admixtures of DXE (4 mM) and repaglinide (6 mM) on motility (solid lines) and $[Ca^{2+}]_{is}$ (broken lines) of human ejaculated sperm in the presence of extracellular Ca^{2+}.

Effect of drug combinations: potentiation of spermicidal activity and $[Ca^{2+}]_{is}$

DBZ (2 mM) and MZE (0.01 mM) are reported to produce complete loss of sperm motility, accompanied by an increase in $[Ca^{2+}]_{is}$ in ejaculated human sperm cells in 60 min (Patni et al 2001; Gulati et al 2006). These concentrations of DBZ and MZE were deliberately chosen so that the effect of their combination with other drugs on motility and $[Ca^{2+}]_i$ could easily be discernible from their effect alone. GBE (6 mM), GLE (6 mM) or RGE (5 mM) produced complete loss of sperm viability in 55 min, 65 min and 50 min, respectively (Figure 1). However, addition of solution containing an admixture of GBE (6 mM), GLE (6 mM) or RGE (5 mM) and DBZ (2 mM) produced complete loss of sperm viability in 15 min in normal BWW medium (Table 1), which was significantly ($P < 0.05$) less compared with the effect of these drugs alone. Further, the time required to produce complete loss of sperm viability by these drug admixtures in the presence of normal BWW medium was observed to be significantly ($P < 0.05$) lower as compared with that in Ca^{2+}-free BWW medium. The time required in the presence of Ca^{2+}-free BWW medium was found to be approximately equal to the time required by DBZ (2 mM) alone (Table 1). Moreover, the critical $[Ca^{2+}]_{is}$ threshold was achieved in significantly ($P < 0.05$) less time when ejaculated spermatozoa were incubated in solution containing these admixtures in the presence of normal BWW medium. This effect was significantly ($P < 0.05$) delayed in the absence of extracellular calcium.

Hence, a synergism was observed for both the time required to produce complete loss of sperm viability and the time required to achieve the critical $[Ca^{2+}]_{is}$ threshold (Table 1). This can be ascribed to simultaneous blockade of K_{ATP} channels by GBE, GLE or RGE, which caused membrane depolarization, subsequent opening of voltage-gated calcium channels, resulting in calcium influx on the one hand and prevention of Ca^{2+} efflux from sperm cells due to blockade of a Na^{+}–Ca^{2+} exchanger on the other hand (Patni et al 2001). It is important to note that the time required for complete loss of sperm motility was significantly ($P < 0.05$) increased in Ca^{2+}-free BWW medium. This was probably due to the requirement of Ca^{2+} influx as a prerequisite for sulfonylureas and meglitinide analogues to produce spermicidal action. These findings, together with the fact that the spermicidal activity of DBZ resides in its ability to block efflux of $[Ca^{2+}]_{is}$ and is independent of extracellular calcium, suggest that the spermicidal action observed in the absence of extracellular calcium was due only to DBZ and not due to the effect of other drugs.

Similar results were obtained for sperm motility and $[Ca^{2+}]_{is}$ when GBE (6 mM), GLE (6 mM) or RGE (5 mM) was admixed with MZE (0.01 mM). Table 2 shows that addition of solution containing an admixture of GBE (6 mM), GLE (6 mM) or RGE (5 mM) and MZE (0.01 mM) produced complete loss of sperm viability in 15 min in normal BWW medium. This duration was significantly ($P < 0.05$) shorter compared with that required by similar concentrations of GBE (6 mM), GLE (6 mM) or RGE (5 mM) and MZE (0.01 mM) alone. The shorter duration required to produce complete loss of sperm viability by these

Table 1 Influence of solution containing an admixture of 2′,4′-dichlorobenzamil hydrochloride (DBZ; 2 mM) and glibenclamide (GBE; 6 mM), gliclazide (GLE; 6 mM) or repaglinide (RGE; 5 mM) on motility and $[Ca^{2+}]_{is}$ of ejaculated human sperm in normal BWW or Ca^{2+}-free BWW medium

Incubation medium	Time (min)	DBZ + GBE		DBZ + GLE		DBZ + RGE	
		FM	$[Ca^{2+}]_{is}$ (nM)	FM	$[Ca^{2+}]_{is}$ (nM)	FM	$[Ca^{2+}]_{is}$ (nM)
Normal BWW	2	0.87 ± 0.03	598.36 ± 29.76	0.84 ± 0.05	579.68 ± 27.89	0.83 ± 0.03	687.58 ± 31.25
	5	0.69 ± 0.0	876.38 ± 49.68	0.67 ± 0.03	761.39 ± 35.72	0.62 ± 0.01	928.65 ± 50.27
	10	0.28 ± 0.01	1123.68 ± 56.46	0.25 ± 0.02	1065.32 ± 53.46	0.24 ± 0.01	1155.87 ± 55.91
	15	0	1157.89 ± 57.89	0	1130.58 ± 54.81	0	1173.52 ± 56.34
	20		1185.63 ± 58.92		1183.15 ± 58.35		1180.89 ± 57.29
	25				1202.37 ± 60.15		
Ca^{2+}-free BWW	2	0.98 ± 0.06	113.27 ± 8.97	0.99 ± 0.07	105.67 ± 7.54	0.98 ± 0.06	120.58 ± 8.37
	5	0.94 ± 0.04	187.69 ± 10.36	0.96 ± 0.03	179.62 ± 9.33	0.94 ± 0.05	185.42 ± 11.13
	10	0.91 ± 0.03	215.79 ± 14.59	0.87 ± 0.02	219.87 ± 12.58	0.85 ± 0.03	231.56 ± 13.56
	15	0.84 ± 0.04	289.19 ± 15.19	0.84 ± 0.05	263.98 ± 13.43	0.73 ± 0.02	265.39 ± 14.18
	20	0.78 ± 0.05	436.73 ± 22.28	0.81 ± 0.04	359.26 ± 20.57	0.67 ± 0.01	412.37 ± 21.37
	25	0.69 ± 0.03	513.27 ± 25.76	0.72 ± 0.04	415.37 ± 22.15	0.60 ± 0.02	489.67 ± 24.98
	30	0.65 ± 0.02	574.65 ± 30.94	0.66 ± 0.03	489.79 ± 25.76	0.53 ± 0.03	567.19 ± 31.23
	35	0.60 ± 0.04	638.97 ± 35.12	0.58 ± 0.04	577.19 ± 30.73	0.41 ± 0.02	634.52 ± 34.56
	40	0.52 ± 0.03	701.32 ± 38.21	0.51 ± 0.03	619.35 ± 32.61	0.35 ± 0.04	698.73 ± 37.14
	45	0.40 ± 0.02	768.15 ± 41.03	0.45 ± 0.02	754.28 ± 33.47	0.25 ± 0.03	763.57 ± 40.89
	50	0.21 ± 0.01	867.28 ± 53.67	0.31 ± 0.03	849.83 ± 43.58	0.12 ± 0.01	863.21 ± 52.36
	55	0.11 ± 0.02	1016.34 ± 56.34	0.26 ± 0.02	975.52 ± 52.87	0	1025.64 ± 55.89
	60	0	1120.39 ± 58.23	0.12 ± 0.03	1089.28 ± 56.18		1145.32 ± 59.26
	65		1145.28 ± 60.76	0	1167.78 ± 59.79		1189.37 ± 60.23
	70				1193.16 ± 61.24		1201.39 ± 61.43

FM, fractional motility. Blank rows indicate termination of experiment after observing 100% immotility. Values of FM and $[Ca^{2+}]_{is}$ are expressed as mean ± s.d of six volunteers.

admixtures could be ascribed to influx of Ca^{2+} due to blockade of K_{ATP} channels by sulfonylureas and meglitinide analogues, and simultaneous inhibition of endoplasmic reticulum Ca^{2+}-ATPase by MZE, which prevented the reuptake of cytosolic Ca^{2+} by intrasperm Ca^{2+} stores. However, this effect was significantly ($P < 0.05$) delayed in Ca^{2+}-free BWW medium (Table 2). This was probably due to negligible Ca^{2+} influx in Ca^{2+}-free BWW medium and elevation of $[Ca^{2+}]_{is}$ was only due to inhibition of endoplasmic reticulum Ca^{2+}-ATPase by MZE, which prevented the reuptake of cytosolic Ca^{2+} by intrasperm Ca^{2+} stores.

Conclusion

The addition of GBE, GLE or RGE to ejaculated human semen samples produced a marked increase in $[Ca^{2+}]_{is}$. This effect was accompanied by a reduction in sperm viability. However, the time taken to achieve the critical $[Ca^{2+}]_{is}$ threshold was approximately 2-fold greater in the presence of Ca^{2+}-free BWW medium. Hence, the Ca^{2+} influx from an extracellular source played a major role in the spermicidal action of these drugs. The addition of DXE, a K_{ATP} channel opener, to solutions containing GBE, GLE or RGE resulted in prolongation of the time taken to produce complete loss of ejaculated sperm viability. This strongly suggested that the spermicidal activity of these drugs was due to their K_{ATP} channel-blocking property. Furthermore, the spermicidal activity of these drugs was found to be potentiated by DBZ (a Na^+–Ca^{2+} exchange inhibitor) as well as by MZE (a Ca^{2+}-ATPase inhibitor), respectively, due to inhibition of cytosolic Ca^{2+} efflux and prevention of reuptake of Ca^{2+} into the calcium stores. Overall, the results of the present investigation revealed a novel spermicidal activity of GBE, GLE and RGE due to their ability to elevate $[Ca^{2+}]_{is}$. Targeting the Ca^{2+}-homeostasis in ejaculated sperm cells for contraception purpose has a clear advantage over currently available spermicidal agents (e.g. nonoxynol-9 and cetrimide). This is because the surfactants have a non-specific action on all the cells and their frequent use is known to alter the vaginal cytology and vaginal membrane permeability, thus increasing the risk of STD/HIV infection (Stafford et al 1998). Further, the potentiation of spermicidal activity of GBE, GLE or RGE by DBZ or MZE indicates the possibility of using other agents affecting Ca^{2+} homeostasis for reducing the spermicidal dose of respective drugs while formulating a contact spermicidal formulation.

Table 2 Influence of solution containing admixture of miconazole (MZE; 0.01 mM) and glibenclamide (GBE; 6 mM), gliclazide (GLE; 6 mM) or repaglinide (RGE; 5 mM) on motility and $[Ca^{2+}]_{is}$ of ejaculated human sperm in normal BWW or Ca^{2+}-free BWW medium

Incubation medium	Time (min)	MZE + GBE		MZE + GLE		MZE + RGE	
		FM	$[Ca^{2+}]_{is}$ (nM)	FM	$[Ca^{2+}]_{is}$ (nM)	FM	$[Ca^{2+}]_{is}$ (nM)
Normal BWW	2	0.85 ± 0.03	598.43 ± 29.75	0.86 ± 0.03	576.49 ± 28.74	0.81 ± 0.03	664.57 ± 31.25
	5	0.64 ± 0.02	965.87 ± 49.78	0.59 ± 0.02	735.87 ± 48.56	0.65 ± 0.02	986.75 ± 52.47
	10	0.26 ± 0.02	1110.84 ± 54.62	0.26 ± 0.01	1016.39 ± 53.79	0.22 ± 0.01	1123.16 ± 55.19
	15	0	1145.63 ± 57.19	0	1130.48 ± 56.43	0	1143.57 ± 56.79
	20		1163.12 ± 59.03		1158.79 ± 58.19		1156.41 ± 58.47
	25				1176.53 ± 59.31		
Ca^{2+}-free BWW	2	1.01 ± 0.05	114.97 ± 6.13	0.99 ± 0.04	99.43 ± 7.49	1.03 ± 0.06	107.58 ± 5.98
	5	0.98 ± 0.03	189.16 ± 9.45	0.97 ± 0.05	148.76 ± 9.87	1.01 ± 0.03	167.34 ± 8.76
	10	0.95 ± 0.04	234.34 ± 11.76	0.94 ± 0.03	226.39 ± 12.15	0.97 ± 0.05	224.63 ± 11.46
	15	0.90 ± 0.03	289.17 ± 13.98	0.91 ± 0.03	291.45 ± 14.05	0.95 ± 0.02	287.14 ± 14.06
	20	0.85 ± 0.02	365.48 ± 18.45	0.87 ± 0.03	368.72 ± 17.86	0.91 ± 0.02	361.29 ± 19.38
	25	0.81 ± 0.02	438.19 ± 20.76	0.81 ± 0.01	443.55 ± 19.45	0.87 ± 0.01	431.17 ± 21.67
	30	0.74 ± 0.03	479.38 ± 22.47	0.76 ± 0.02	486.48 ± 21.97	0.82 ± 0.03	482.07 ± 23.16
	35	0.71 ± 0.04	592.36 ± 25.79	0.72 ± 0.05	583.26 ± 24.56	0.77 ± 0.05	585.42 ± 26.75
	40	0.63 ± 0.03	639.78 ± 28.96	0.64 ± 0.03	648.55 ± 27.83	0.70 ± 0.04	629.63 ± 29.48
	45	0.48 ± 0.03	738.55 ± 32.15	0.61 ± 0.03	742.59 ± 31.71	0.61 ± 0.03	749.81 ± 31.13
	50	0.35 ± 0.03	836.26 ± 37.98	0.44 ± 0.02	847.16 ± 36.96	0.45 ± 0.03	856.46 ± 38.78
	55	0.16 ± 0.01	935.41 ± 44.58	0.18 ± 0.01	938.29 ± 43.28	0.31 ± 0.02	968.79 ± 45.33
	60	0	1101.49 ± 52.34	0	1120.96 ± 51.43	0.13 ± 0.01	1076.32 ± 51.19
	65		1145.43 ± 56.05		1148.37 ± 55.29	0	1157.84 ± 55.63
	70		1163.18 ± 57.49		1167.18 ± 58.47		1179.31 ± 58.12

FM, fractional motility. Blank rows indicate termination of experiment after observing 100% immotility. Values of FM and $[Ca^{2+}]_{is}$ are expressed as mean ± s.d of six volunteers.

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