J. Pharm. Pharmacol. 1992, 44: 534-536
Communicated May 10, 1991

Effects of lithium and haloperidol on human sperm motility in-vitro

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Abstract—Two psychotropic drugs, lithium and haloperidol, were evaluated for their in-vitro effects on sperm motility using a trans-membrane migration method. Sperm motility was measured either immediately after semen had been mixed with the drug or after a 2 h incubation period at 37°C. Lithium inhibited human sperm motility in a dose-dependent manner with an EC50 of 10 mM when the semen–lithium mixture had been incubated. Sperm motility was increased to 127% of control when semen had been incubated with 0·027 μM haloperidol; this concentration was within the therapeutic range.

Lithium is a widely prescribed drug used for the treatment and prophylaxis of manic depressive psychosis. Although the therapeutic usefulness of lithium is undisputed, a wide range of adverse effects on metabolic and endocrine functions has been demonstrated following lithium treatment (Vinarova et al 1972; Banerji et al 1982; Koch & Peters 1987). However, the effect of lithium on the reproductive system, especially on sperm motility, has received little attention. Other psychotropic drugs, such as chlorpromazine and imipramine, have been found to have an inhibitory effect on human sperm motility in-vitro (Levin et al 1981; Hong et al 1982, 1984).

Sperm motility is one of the important parameters of sperm function. Immotile sperm, whether dead or living, cannot penetrate the cervical mucus and fertilize the ovum (Tampion & Gibbons 1963; Blandau & Rumery 1964). The present study focuses on the in-vitro effect of two psychotropic drugs, lithium and haloperidol, on human sperm motility.

Materials and methods

Fresh human semen samples were collected from healthy donors. Only semen samples with sperm concentration higher than 15 million mL^{-1} and the percentage of progressive forward moving sperm higher than 20% were used. A series of our previous studies have shown that these values were minimal essential criteria for the trans-membrane method (Hong et al 1981, 1982; Shen et al 1991). Standard powders of lithium and haloperidol were purchased from Sigma, USA. The tested drugs were dissolved in phosphate buffered saline (Dulbecco 'A', Oxoid Ltd, pH 7·3). Each semen sample was divided into several 100 μL portions which were then mixed with 50 μL of drug solution or phosphate-buffered saline. The motility of semen in buffer was used as control and those of semen in drug mixtures were expressed as a percentage of control.

A trans-membrane migration method was used to measure sperm motility in each tube. In order to assess the drug effect of different exposure time on sperm motility, sperm motility was measured either immediately after semen had been mixed with drug or after a 2 h incubation period at 37°C. The significance of the 2 h incubation is to investigate the drug effect on capacitation and acrosome reaction of sperm, which occurs 2 h after ejaculation and which is required for the penetration of zona pellucida and fusion with the oocyte (Yanagimachi 1988). One hundred μL of the semen–drug or semen–buffer mixture was pipetted into the upper chamber. The proportion of sperm that moved across the 5 μm pores of a Nuclepore membrane (Nuclepore, USA) from the upper chamber into the lower chamber containing phosphate-buffered saline, during a 2 h incubation at 37°C, was called the trans-membrane migration ratio (TMMR) (Hong et al 1981). Previous studies have shown that the TMMR is a quantitative and reproducible parameter for sperm motility (Hong et al 1981, 1984; Gadd & Curtis-Prior 1988; Shen et al 1991).

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Results

Fig. 1 shows the concentration-response curve for the inhibitory effect of lithium on human sperm motility. For the semen-drug mixture, the EC50 was 10 mM. Without incubation, sperm motility could not be inhibited by lithium (up to 13 mM) to less than 50% of control.

Fig. 2 shows the log concentration-dependent effect of haloperidol on human sperm motility. Without incubation, there was no statistically significant difference in sperm motility at any concentration ($P > 0.05$, analysis of variance). However, sperm motility could be increased if semen had been incubated for 2 h with 0·027 μM haloperidol (10 ng mL^{-1}) ($P < 0.01$).

Discussion

In this study, we have shown that lithium inhibited human sperm motility. There are few reports on the in-vitro effect of lithium on sperm motility and their results are conflicting. Macleod et al (1949) found that lithium, in relatively small concentrations, inhibited the metabolism and motility of human spermatozoa. Both aerobic and anaerobic pathways of glycosis were affected. There was a concentration-dependent inhibition of sperm

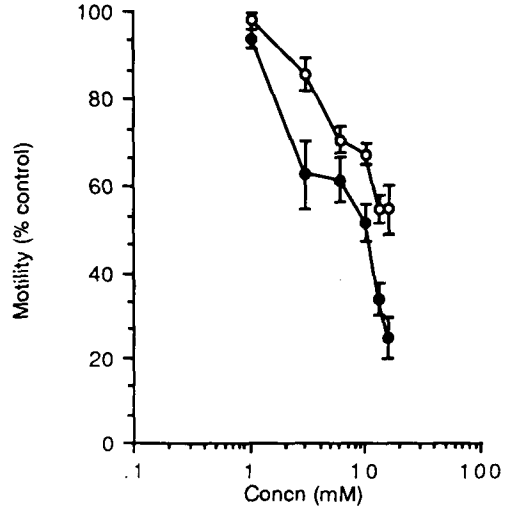

FIG. 1. Log concentration-response curves for inhibitory effects of lithium carbonate on human sperm motility. Semen had been incubated with lithium carbonate for 0 (○) and 2 h (●) before sperm motility was determined using a trans-membrane migration method. All points are mean ± s.e.m. of 7 different samples. Motility of sperm in semen samples mixed with phosphate-buffered saline was used as control.

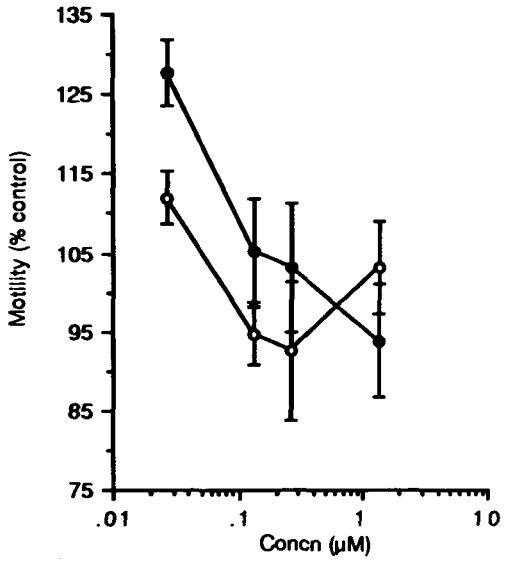

FIG. 2. Log concentration-response curves for the effects of haloperidol on human sperm motility. Semen samples had been incubated with haloperidol for 0 (○) and 2 h (●) before trans-membrane migration. Each point represents mean ± s.e.m. of 7 different samples. Motility of sperm in semen samples mixed with phosphate-buffered saline was used as control.

motility with almost complete immobilization of motility at a concentration of 25 mM. Gibbons & Gibbons (1984) found that 6–8 mM lithium could cause complete cessation of flagellar movement in sea urchin sperm. This immobilizing effect of lithium was completely reversed by 100-fold dilution with fresh solution containing no lithium. Gibbons & Gibbons (1984) suggested that lithium may act on the regulatory site through which calcium modulated the asymmetry of flagellar movement. However, another in-vitro study, using a turbidimetric method, demonstrated no significant effect of lithium on sperm motility (Levin et al 1981). In-vivo studies on the effect of lithium on sperm motility and male fertility are equally conflicting. Kolomaznick et al (1981) reported significantly lower spermatogram findings in terms of sperm count and motility of samples obtained from 9 patients on lithium and neuroleptic drugs. However, Raboch et al (1981) found no abnormality in sperm count, motility or morphology of semen samples obtained from 10 patients on lithium therapy.

Lithium is widely distributed throughout body tissues when given orally and concentrations of lithium in semen after oral administration are about double those in blood (Raoof 1988). Values for lithium concentrations as high as 3·2 mM have been observed in semen of some healthy volunteers treated with therapeutic doses of lithium. From our present results, this value will correspond to a reduction of about 20–35% in sperm motility. It is possible that lithium may affect sperm motility in some patients receiving chronic lithium therapy. The present findings suggest that further studies on patients receiving lithium therapy are needed, using objective measurements of sperm motility in which semen quality in terms of sperm count, motility and morphology should be studied before and after starting lithium treatment.

It is interesting to find that haloperidol, a calmodulin antagonist (Godfraind 1987), has a stimulatory effect on human sperm motility during the processes of capacitation and acrosome reaction of sperm. In a previous study (Hong 1989), trifluperazone, another calmodulin antagonist, was also found to stimulate human sperm motility. Spermatozoa contain high concentrations of calmodulin (Brokaw & Nagayama 1985) and calmodulin-dependent phosphorylation has been suggested as a mechanism whereby calcium could negate the positive effect of cyclic AMP on sperm motility (Wasco & Orr 1984). When sperm motility in semen-drug mixture was evaluated with pre-incubation, the amplitude of maximal motility increase induced by haloperidol was 27% only. Compared with other motility-stimulating substances which have been evaluated with the trans-membrane migration method (Hong 1989), the stimulatory effect of haloperidol was relatively weak. However, it should be emphasized that 0·027 μM haloperidol is within the therapeutic serum levels of this drug.

In conclusion, the present study showed that lithium inhibited human sperm motility in-vitro in a dose-dependent manner and haloperidol increases sperm motility when semen has been incubated with 0·027 μM haloperidol.

This study was supported by a Summer Student Grant from the National Science Council, Republic of China (NSC-79037-008). We are indebted to Dr C. Y. Hong for advice on the sperm motility assay and helpful discussion.

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J. Pharm. Pharmacol. 1992, 44: 536

Book Review

Peptide and Protein Drug Delivery
Edited by Vincent H. L. Lee
Published 1991 Marcel Dekker Inc., New York
912 pages
ISBN 0 8247 7896 0 $150.00 USA and Canada, $180.00 all other countries

One sign of a good textbook is the number of colleagues who want to borrow it: an even greater endorsement is the length of time it takes to get it back. On the basis of both these criteria, this is a very good book and the major reason for the length of time that it took to write this review was the difficulty in keeping it on my desk long enough to read it.

The book is the fourth in a series on *Advances in Parenteral Sciences* and it has to be said that it is a volume which does not fall comfortably under the general series title. The fundamental topics that are covered include peptide and protein synthesis, physical chemistry and biochemistry, analysis, enzymatic and membrane constraints and pharmacokinetics. Comprehensive reviews of delivery by parenteral, oral, buccal, rectal, nasal, vaginal and transdermal routes are also included, together with background information on anatomy, biochemistry and physiology relevant to such sites of delivery. A final section includes details of mucoadhesion, formulation, controlled release, toxicity, immunogenicity and regulatory aspects. It is obvious that the series title would not naturally lead the enquirer to this excellent volume.

As with most volumes nowadays, this one is multi-authored which does of course mean that the editor must select his titles and authors in advance. With a subject like peptide and protein delivery, this approach could be dangerous since progress in science may well outpace the authors. I was very pleased to see that the editor had not only realized this fact but had also taken the responsibility himself to cover, in the first chapter of the book, those areas where major developments had occurred.

It would be impossible to review all 22 chapters of the book and I shall not attempt this task. In general, an excellent volume was slightly let down by the quality of some of the diagrams, the photographs were of little use and there were inconsistencies in some of the references. On a more positive note, each chapter contained an extensive bibliography which would provide any reader with an excellent introduction to each topic.

The editor has grouped the chapters under three headings covering (a) the fundamentals of peptide and protein delivery, (b) a review of the approaches taken to optimise absorption from the various routes and (c) the practical considerations relating to the formulation and registration of delivery systems. In the first section, the chapters are all well written and comprehensive, containing a wealth of practical information and details of techniques. However, none of the authors have been afraid to interject some basic definitions to remind the reader of important points. A good example is the differentiation between a peptide and protein in the chapter on the physical biochemistry of protein drugs. Each of the authors has been very careful to ensure that overlap has not occurred and there is a refreshing lack of repetition, which is not always the case in volumes of this type.

A major barrier which must be overcome if a peptide or protein is to be delivered successfully is that presented by enzymatic activity which can occur at the site of administration and can be extensive. A consequence of this is that even relatively small peptides, such as thyrotrophin-releasing hormone, a tripeptide, produce bioavailabilities of 67·5 and 31·1% respectively when given by the subcutaneous and intramuscular routes. The excellent chapter which deals with this aspect covers the characteristics of the enzymes, their distribution in tissues and cells and structure/activity relationships. It is followed by a review of the nature of the degradation of specific examples and means of its prevention. However, one of the problems with the timescale that is involved in publication is that events can outstrip the text. The chapter in question ends with a mention of the work which attempted the oral delivery of insulin by a formulation imitating chylomicra. Although the authors were correct in posing a number of sceptical questions, the subsequent admission that the formulations were contaminated with an oral hypoglycaemic agent was not a possibility, quite reasonably, that the reviewers contemplated.

It is, of course, quite reasonable that the first section occupies almost half of the volume and the seven chapters on delivery about 50% of this space. Examples of successful delivery systems are limited and the authors in the section have had a smaller literature to deal with. The important common feature to delivery by any of the routes considered is, often, insufficient bioavilability which is subject to great fluctuation. As a consequence, some of the routes could only be included for their potential rather than their achievement. The conclusions to the chapter on transdermal delivery of peptides provide a succinct summary relating to this point.

The final section is the smallest and least coherent but again provides balanced and authoritative reviews. It will doubtless provide the type of information which it is easy to question and the authors are to be congratulated for their perception and courage.

I like this book. It is extensive (912 pages), it is weighty (1·74 kg) and it is relatively inexpensive ($150 in the USA, £103 in the UK). At about 10p per page it is much better value than a telephone call, since to read a page takes longer and is much more rewarding: purchasers would not feel cheated if no change is given.

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