Br. J. clin. Pharmac. (1991), **31**, 711–714 ADONIS 030652519100129D

Pentoxifylline stimulates human sperm motility both *in vitro* and after oral therapy

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Pentoxifylline is a haemorrheologic agent often used in the treatment of peripheral vascular disorders. In this study, we measured sperm motility with a trans-membrane migration method and investigated the effect of this drug in the treatment of male infertility. We found that pentoxifylline increased motility of ejaculated spermatozoa *in vitro* from both normal and asthenozoospermic samples. After giving pentoxifylline to patients with asthenozoospermia for 3 months, sperm motility significantly increased, but sperm concentration did not increase. From the above results, it can be concluded that pentoxifylline is a useful drug in the treatment of normogonadotropic asthenozoospermia.

Keywords pentoxifylline sperm motility infertility

Introduction

The quality of ejaculated semen is one of the major roles in male fertility. It has been assessed according to the parameters of morphology, concentration, and motility, since *in vivo* conception was assumed to occur only if there was a certain critical number of morphologically normal, motile sperm in the ejaculate (Coetzee *et al.*, 1990). Furthermore, poor sperm motility is considered a more likely reason for infertility than either a low total count or increased number of spermatozoa with abnormal morphology (Cai & Marik, 1989).

Pentoxifylline is an orally active haemorrheologic agent used in the treatment of intermittent claudication and other vascular disorders (Porter *et al.*, 1982; Ward & Clissold, 1987). It is a methylxanthine derivative and can increase intracellular cAMP levels by inhibiting phosphodiesterase (Grigoleit *et al.*, 1976). In light of the fact that methylxanthine derivatives could increase the duration of the activity of ejaculated spermatozoa (Schoenfeld *et al.*, 1975), we investigated the *in vitro* effect of pentoxifylline on the motility of normal and asthenozoospermic sperm with a trans-membrane migration method. In addition, we studied the effect of pentoxifylline therapy on oligo- or asthenozoospermic patients.

Methods

In vitro *drug study*

Semen samples were collected from seven normal volunteers and nine asthenozoospermic patients. The diagnosis of asthenozoospermia was based on the exclusion of signs related to infectious, endocrine or immunological diseases. All normal samples had concentrations higher than 20 million spermatozoa ml^{-1} and more than 20% of progressive forward motility. Sperm concentration in asthenozoospermic subjects ranged from 15 to 60 million ml^{-1}. All semen samples were collected by masturbation after 3 days of sexual abstinence and were examined within 1 h after ejaculation. A standard powder of pentoxifylline was obtained from Hoechst, FRG. Five drug concentrations (0.036, 0.18, 0.36, 1.8, 3.6 μM) were prepared by dissolving standard powder in phosphate buffered saline (Dulbecco 'A', pH 7.3).

A trans-membrane migration method was used to examine the *in vitro* effect of pentoxifylline on sperm motility. This method received partial validation against time exposure photomicrography as far as percentage of motility and amplitude of lateral sperm displacement is concerned (Ratnasooriya & Aitken, 1989). Each semen sample was divided into several 100 μl aliquots, which were then mixed with 50 μl of either drug solution or phosphate buffered saline. Semen-drug or semen-buffer mixture (100 μl) were 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). Previous studies have shown that the TMMR is a quantitative and reproducible parameter for sperm motility (Hong *et al.*, 1981; Raoof *et al.*, 1989). Sperm motility in semen mixed with phosphate buffered saline was used as a control and the motility of drug-treated sperm was expressed as a percentage of the control.

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In vivo *study*

Eight infertile men (ages from 28 to 37 years old), with normal androgenic function and at least 3 years of barren marriage, volunteered for the *in vivo* study of pentoxifylline. None of these subjects had any history of endocrine, immunological of infectious diseases. In each subject at least three semen samples were examined over a 3 month interval before this study. Asthenozoospermia (motile sperm less than 50% of total) was demonstrated in all patients ($n = 8$) and six patients also showed oligospermia (sperm number less than 20 million ml^{-1}). All subjects were treated with pentoxifylline (Trental, Hoechst) 800–1200 mg day^{-1} orally. Semen analysis was performed by the same technician before and after 3 months of treatment. All semen samples were obtained by masturbation after 3 days of sexual abstincence. Sperm count was performed within 1 h after collection. Sperm motility (percentage of mobile spermatozoa) was evaluated 1 and 2 h after ejaculation on fresh smears at the optic microscope (magnification, x 400).

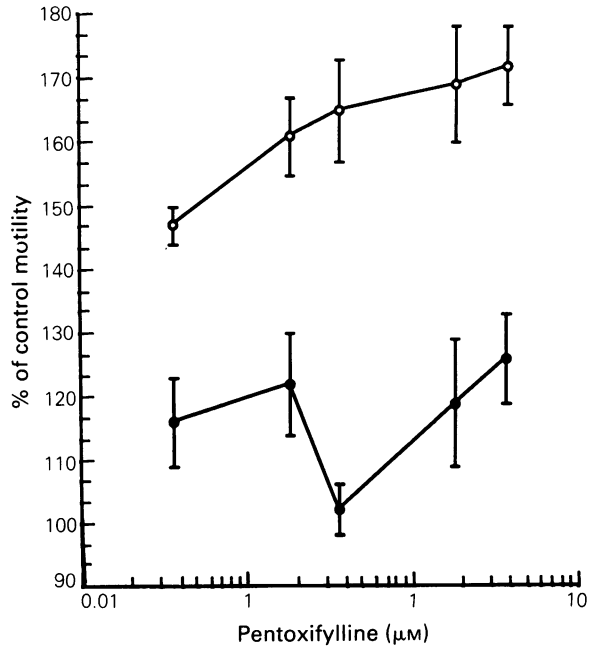

Figure 1 Log concentration-response curves for the effect of pentoxifylline on sperm motility of both normal men (○, $n = 7$) and asthenozoospermic patients (●, $n = 9$). Each point represents mean ± s.e. mean.

Results

Figure 1 shows the log concentration-response curves for the effects of pentoxifylline on sperm motility of both normal and asthenozoospermic samples. Pentoxifylline significantly increased sperm motility in both normal ($P < 0.01$, ANOVA) and asthenozoospermic subjects ($0.01 < P < 0.05$, ANOVA).

The result of *in vivo* study is shown in Table 1. After 3 months of pentoxifylline administration, sperm motility significantly increased ($P < 0.05$, Wilcoxon matched-pairs signed-ranks test), whereas sperm concentration did not alter significantly ($P > 0.05$). Pregnancy was achieved in case G.

Discussion

This study demonstrated that pentoxifylline increases the *in vitro* motility of ejaculated spermatozoa collected from both normal and asthenozoospermic men. This result is compatible with previous studies, which found that drugs with phosphodiesterase-inhibitory activity may increase sperm motility (Schoenfeld *et al.*, 1975). Other *in vitro* studies also showed that pentoxifylline, at concentrations ranging from 0.3 mM to 0.6 mM, prolonged the duration of activity in ejaculated spermatozoa from

Table 1 *In vivo* study of the effectiveness of pentoxifylline on sperm motility of idiopathic infertile patients

	Before therapy			*After 3 month therapy*		
Cases	*Concentration* (10^6 ml^{-1})	*Sperm motility* 1 h	2 h	*Concentration* (10^6 ml^{-1})	*Sperm motility* 1 h	2 h
A	13	30%	27%	21	41%	36%
B	6	25%	20%	13	70%	50%
C	0.3	20%	10%	12	45%	30%
D	7	23%	14%	21	50%	40%
E	32	9%	–	18	60%	45%
F	11	20%	15%	6	70%	60%
G	20	38%	16%	49	70%	65%
H	2	25%	11%	23	53%	35%
Mean	12.8	24%	16%	20.4	57%*	45%*
s.d.	11.4	8%	6%	12.9	12%	12%
n	8	8	7	8	8	8

* Before therapy *vs* after 3 month therapy, $P < 0.05$, by Wilcoxon matched-pairs signed-ranks test.

both normal and asthenozoospermic men (Aparicio *et al.*, 1980; Turner *et al.*, 1978). However, Yovich *et al.* (1988) reported that pentoxifylline significantly improved sperm motility in oligospermic samples, but no difference in motility was noted when pentoxifylline was added to normospermic samples.

Our *in vivo* study showed that pentoxifylline improved sperm motility but did not significantly improve sperm concentration. Other reports on the effect of pentoxifylline in the treatment of oligoor asthenozoospermia were conflicting. Heite (1979) found that this drug may significantly improve both sperm count and motility in oligo-asthenozoospermic patients. Furthermore, Aparicio *et al.* (1980) reported that pentoxifylline increased sperm motility without affecting sperm number in asthenozoospermic subjects. However, Wang *et al.* (1983) could not find any effect of this drug on sperm number and motility in 11 men with idiopathic oligospermia.

The maximal amplitude of motility increase induced by pentoxifylline *in vitro* was 71%. Compared with other motility stimulating substances which had been evaluated with the trans-membrane migration method, the stimulating effect of pentoxifylline was relatively strong. Among the *in vitro* stimulators of human sperm motility which Hong *et al.* have proved, only five stimulators can induce an amplitude of motility increase more than 50% of control (Hong, 1989). And all these five stimulators involve calcium ions: EDTA and EGTA are calcium chelators (Hong *et al.*, 1984), diltiazem and lanthanum chloride are calcium antagonists (Hong *et al.*, 1985a), while caffeine modifies calcium transport across sperm membrane (Hong *et al.*, 1985b). It was concluded that in ejaculated human semen, calcium is detrimental for sperm motility. However, it is little known for the present whether the stimulatory effect of pentoxifylline involves calcium ions. The stimulatory effect of pentoxifylline on sperm motility might be due to its pharmacological action on cAMP metabolism. The possible mechanism for its stimulating effect *in vivo* is as follows. When taken orally and secreted in the seminal fluid (Ward & Clissold, 1987), pentoxifylline and its metabolites will appear in the semen. They inhibit phosphodiesterase and increase cAMP concentrations in the ejaculated spermatozoa thereby improving their motility. Marrama *et al.* (1985) found that pentoxifylline therapy increased fructose concentrations in seminal fluid and decreased sperm ATP levels in patients with oligo-asthenozoospermia. An increased conversion of ATP into cAMP might explain why ATP was reduced.

Most infertile men have either a reduced number of sperm, a large percentage of immotile sperm, a high proportion of abnormal shaped sperm, or any combination of these findings. Among semen parameters, sperm number and morphology are closely related to spermatogenesis and thus difficult to be modified once sperm is formed. Sperm motility is more accessible to pharmacological manipulation even if semen has been ejaculated. Sperm motility is important for penetration through the zona pellucida of ovum and this function has a high correlation with fertilization rates *in vitro* (Bedford, 1982). The practice of mixing sperm with a motility stimulating agent for improving the successful rate of artificial insemination has been reported. Yovich *et al.* (1988) demonstrated that pentoxifylline increased the successful rates of *in vitro* fertilization in cases of oligo- or asthenozoospermia.

In conclusion, we found that pentoxifylline stimulated sperm motility both *in vitro* and *in vivo*. This drug could be useful in the treatment of normogonadotropic asthenozoospermia.

This study was supported by a Summer Student Award from the National Science Council, Republic of China (NSC-80037-008). We are indebted to Ms Tsan-Ju Chen for statistical and graphic help.

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(Received 2 October 1990, accepted 11 January 1991)