

Effects of pentoxifylline on sperm motility characteristics of normozoospermic semen using computer-assisted semen analysis (CASA) : a preliminary report.

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Computer-assisted sperm movement analysis was used to study the effect of pentoxifylline on human sperm motility characteristics of normozoospermic semen. The study focused on the following issues : the changes in individual movement characteristics in response to pentoxifylline, the persistence of the response during drug treatment. Computerized analysis was started at 30 minutes, 3 hours and 24 hours, after addition of pentoxifylline. Data obtained showed that pentoxifylline had no significant effect on the percentage of motile sperm, percentage of progressive motile sperm, average path velocity (VAP), linearity (LIN), straightness (STR), and beat cross frequency (BCF). The straight line velocity (VSL) significantly increased at 30 minutes and curvilinear velocity (VCL), amplitude of lateral head displacement (ALH) significantly increased at 3 hours after exposure to the drug, respectively. After exposure to pentoxifylline for 24 hours, the sperm movement characteristics did not differ from corresponding controls. In conclusion, we have shown that pentoxifylline increases the quality of sperm motility in normal men. However, whether this change leads to an increase in fertilizing ability requires further study.

Key words : Pentoxifylline, Sperm motility characteristics, Normozoospermia, Computer-assisted semen analysis (CASA)

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นเรศ สุขเจริญ, อุษณีย์ เจตน์สว่าง, เย็นจิต จันทร์ประสิทธิ์, จิราพร เหยี่ยมวิจาวัฒน์, เอนก อารี-พรรค. ผลของเพ็นโทซิไพลินต่อลักษณะการเคลื่อนไหวของตัวอสุจิในน้ำอสุจิปกติโดยการตรวจด้วยเครื่องคอมพิวเตอร์: รายงานเบื้องต้น. จุฬาลงกรณ์เวชสาร 2536 กันยายน ; 37(9) : 555-560

ได้ทำการศึกษาผลของ Pentoxifylline ต่อลักษณะการเคลื่อนไหวของตัวอสุจิในน้ำอสุจิปกติโดยการตรวจด้วยเครื่องคอมพิวเตอร์ การศึกษานี้เน้นถึงผลของ Pentoxifylline ต่อลักษณะการเคลื่อนไหวของตัวอสุจิแต่ละแบบ และระยะเวลาการออกฤทธิ์ระหว่างการใช้ยา โดยทำการวิเคราะห์ที่ 30 นาที, 3 ชั่วโมง และ 24 ชั่วโมงหลังจากเติม Pentoxifylline. พบว่า Pentoxifylline ไม่มีผลต่อร้อยละของตัวอสุจิที่เคลื่อนไหว, ร้อยละของตัวอสุจิที่เคลื่อนไหวไปข้างหน้าอย่างรวดเร็ว, Average path velocity (VAP), Linearity (LIN), Straightness (STR), และ Beat cross frequency (BCF) อย่างมีนัยสำคัญ Straight line velocity (VSL) เพิ่มขึ้นอย่างมีนัยสำคัญที่ 30 นาที และ Curvilinear velocity (VCL), Amplitude of lateral head displacement (ALH) เพิ่มขึ้นอย่างมีนัยสำคัญที่ 3 ชั่วโมงภายหลังการเติมยาตามลำดับ ภายหลังได้รับยา 24 ชั่วโมง พบว่าลักษณะการเคลื่อนไหวของตัวอสุจิไม่แตกต่างจากกลุ่มควบคุม โดยสรุป Pentoxifylline เพิ่มคุณภาพการเคลื่อนไหวของตัวอสุจิในชายปกติ อย่างไรก็ตามยังคงต้องการการศึกษาต่อไปเกี่ยวกับผลการเปลี่ยนแปลงการเคลื่อนไหวของตัวอสุจิต่อความสามารถของการเจริญพันธุ์

Sperm motility is considered one of the most important parameters in evaluating the fertilizing ability of semen. Sperm motility is necessary for penetration through the zona pellucida and this function had been shown to have a high correlation with fertilization rates *in vitro*.^(1,2) The recent introduction of computerized sperm motility analysis has allowed detailed studies of sperm movement characteristics.

Various chemical compounds have been reported to stimulate the motility of human spermatozoa *in vitro*, including methylxanthines such as caffeine,⁽³⁻⁵⁾ theophylline,⁽⁶⁾ and pentoxifylline.^(5,7) These agents interfere with the cellular oxidative metabolism by inhibiting cyclic adenosine 3':5' monophosphate (cAMP) phosphodiesterase. This results in an increase in cAMP, which boosts the cellular glycolysis with rising endogenous adenosine triphosphate production, which may enhance flagellar beating in the spermatozoa.^(8,9)

Pentoxifylline (3-7- dimethyl-1-5-oxohexylxanthine) is a phosphodiesterase inhibitor with a longer lasting activity and higher hydrosolubility than caffeine. It was also reported to have a greater stimulatory effect on sperm motility.^(5,10) In addition to selection techniques yielding sperm suspensions enriched in highly motile cells, pentoxifylline has been claimed to improve sperm movement when added to fresh semen.

Encouraging as these preliminary data are, a wider use of pentoxifylline in assisted reproduction will necessitate a better understanding of the drug's effect on sperm movement. Before pentoxifylline becomes accepted as a routine treatment for male factor infertility, it would seem essential to ascertain exactly what characteristics of sperm motility are altered by the drug, over the precise range of times it acts and persistence of the response during drug treatment, using normal sperm as a baseline.

In the present study, we addressed these issues by examining the effects of pentoxifylline on sperm movement characteristics using computer-assisted sperm analysis. Particular attention was paid to determination of the persistence of the response during drug treatment.

To determine the specific action of the drug on sperm motility, we administered it *in vitro* so that observed changes were due to it alone. We also ensured that control and treated groups were treated identically but for the presence of the drug. Semen samples were studied individually to avoid any possible interaction that pooling of samples might have.

Materials and methods

Ten semen specimens were obtained by masturbation after at least 48 hours sexual abstinence from 10 healthy donors. These specimens were classified as normozoospermic according to the criteria defined by the World Health Organization.⁽¹¹⁾ Semen specimens were allowed to liquefy at room temperature for 30 minutes.

Aliquots of liquefied semen was divided into 2 tubes. The specimens were washed twice in standard two-layered percoll gradient (20 minutes at 500 xg). After centrifugation, the pellet of first tube was resuspended with Ham's F-10 (control group) and the pellet of the other tube was resuspended with 3.6 mM pentoxifylline (treatment group). Sperm suspension was divided to three tubes in each group and incubated (37 ° C, 5% CO₂ in air) for different periods of time before movement analysis. The samples of each group were analysed in an integrated visual optical system fully automated computer-assisted semen analyser (Hamilton Thorn Research, Beverly, MA, USA) at 30 minutes, 3 hours and 24 hours, respectively, during exposure to the drug. The settings employed for analysis were : frame acquisition rate (frame/second), 25; minimum contrast, 7; minimum size, 6; low-size gate, 0.4; high-size gate, 1.6; low-intensity gate, 0.4; high-intensity gate, 1.6; HTM magnification factor, 2.00.

The parameters recorded for each sample were as follows : the percentage of motile sperm, the percentage of progressively motile sperm, average path velocity (VAP; the time average velocity of the sperm head as projected along its spatial average trajectory), straight line velocity (VSL; the straight line distance from beginning to end of track divided by time taken), curvilinear velocity (VCL; measure of the total distance travelled by a given sperm during the acquisition divided by the time elapsed), linearity (LIN = VSL/VCL; departure of sperm track from a straight line), straightness (STR=VSL.VAP; the effective linearity of the average path), amplitude of lateral head displacement (ALH; the mean width of sperm head oscillation), the beat cross frequency (BCF; frequency of sperm head crossing sperm average path).⁽¹²⁾

Statistical analysis

In view of the possible nongaussian distribution of the velocities, the nonparametric Wilcoxon signed ranks test was employed to examine the differences between control and treated groups. The analysis was carried out with the use of a statistical program (SPSS-PC + : SPSS, Inc., Chicago IL) and using the raw data from each individual at each time point. Probability values of <0.05 were considered significant.

Results

Pentoxifylline had no significant effect on the percentage of motile sperm, percentage of progressive motile sperm, average path velocity (VAP), linearity (LIN), straightness (STR), and beat cross frequency (BCF). The VSL significantly increased at 30 minutes and VCL, ALH significantly increased at 3 hours after exposure to the drug, respectively. After exposure to pentoxifylline for 24 hours, the sperm movement characteristics did not differ from corresponding controls.

Table 1. Effect of pentoxifylline on sperm motility parameters.**

Sperm motility parameters	Time		
	30 minutes	3 hours	24 hour
Motility (%)			
Control	58.0	52.0	45.0
PF	63.0	59.5	45.0
% Difference	+8.6	+14.4	0.0
p value	0.2622	0.2213	0.8590
Progressive motility (%)			
Control	44.5	41.5	30.5
PF	51.5	45.0	33.0
% Difference	+15.7	+8.4	+8.2
p value	0.8580	0.2066	0.9057
Average path velocity : VIP (microns/second)			
Control	79.0	74.0	51.5
PF	81.5	84.0	54.0
% Difference	+3.2	+13.5	+4.8
p value	0.1141	0.0745	0.7353
Curvilinear velocity : VCL (microns/second)			
Control	87.5	83.0	57.5
PF	94.5	100.0	61.0
% Difference	+8.0	+20.5	+6.1
p value	0.0831	0.0367*	0.9528
Straight line velocity : VSL (microns/second)			
Control	70.5	65.5	47.0
PF	75.5	78.0	50.0
% Difference	+7.1	+19.1	+6.4
P value	0.0367*	0.1029	0.5940
Linearity : LIN = VSL/VCL (%)			
Control	79.5	79.5	79.0
PF	80.5	80.5	81.0
% Difference	+1.3	+1.3	+2.5
p value	0.6103	0.9594	0.3139
Straightness : STR = VSL/VAP (%)			
Control	90.0	89.5	88.0
PF	91.0	90.5	90.0
% Difference	+1.1	+1.1	+2.3
p value	0.8590	0.5940	0.2640
Amplitude of lateral head displacement : ALH (microns)			
Control	3.7	3.8	2.6
PF	4.2	4.1	2.7
% Difference	+13.5	+7.9	+3.8
p value	0.1925	0.0330*	0.9528
Beat cross frequency : BCF (Hz)			
Control	10.95	10.40	9.05
PF	11.00	11.60	10.40
% Difference	+0.45	+11.53	+14.92
p value	0.3081	0.4755	0.9528

PE = Pentoxifylline

* p < 0.05, Wilcoxon signed rank test

** Values are median

Discussion

Pentoxifylline is a phosphodiesterase inhibitor of the methylxanthine group which increases the intracellular concentration of cyclic nucleotides, cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP), by inhibiting nucleotide breakdown.⁽¹³⁾ cAMP is known to be an important regulatory element controlling axonemal motility by its action on protein phosphorylation⁽⁹⁾ and a relationship between the fertilizing ability and cAMP content in human spermatozoa has been reported.⁽¹⁴⁾ Therefore, the effect of pentoxifylline on sperm cAMP levels is probably the principal molecular mechanism underlying the observed changes in sperm movement characteristics.

The main changes in movement characteristics occurring in motile spermatozoa in response to pentoxifylline treatment can be summarized as forward acceleration, augmentation of lateral head excursions and intensification of flagellar beat. These changes in movement pattern clearly increase the consumption of energy. It seems that the vigour of sperm motion is moderated by some energy sparing mechanism which is overcome by the action of pentoxifylline.

Previous studies demonstrated an increase in the percentage of motile spermatozoa after exposure to pentoxifylline.⁽⁷⁾ In our study, nonsignificant trends toward the increased percentage of motile sperm and percentage of progressive motile sperm were noted by the presence of pentoxifylline at any of the times studied. In agreement with Yovich et al, we did not observe any significant effect of pentoxifylline on the concentration of motile spermatozoa in normozoospermic specimens. However, some movement characteristics were affected by the treatment.⁽¹⁵⁾ In another study dealing with human spermatozoa from normal donors, pentoxifylline has also been shown to produce only slight effects on percentage motility, but it significantly increased sperm velocity.⁽¹⁶⁾

Incubation with pentoxifylline in our study, when compared with control, caused a significant increase in VSL at 30 minutes and VCL, ALH at 3 hours after exposure to the drug. It was shown by Hammitt et al that pentoxifylline caused a significant increase in VSL and decreased in LIN.⁽⁵⁾ Sikka and Hellstrom demonstrated that pentoxifylline exposure resulted in an increase in VSL, VCL, and LIN, there being no effect on ALH.⁽¹⁷⁾ In contrast, Rees et al observed that pentoxifylline treatment caused a slight rise in ALH and a significant increase in VSL.⁽¹⁶⁾

In our study, pentoxifylline had an effect on VCL and VSL. Our results would therefore add support to those of Barlow,⁽¹⁸⁾ who showed that improved both VCL and VSL, can be correlated with improved rates of fertilization in in vitro fertilization (IVF). However, our result contrast with those of Lewis et al, who found that pentoxifylline had no effect on the VSL at any times measured.⁽¹⁹⁾ Other workers also found that linearity did not increase with an increase in VSL,⁽²⁾ suggesting an

increase in VCL as well because linearity is $(LIN = VSL/VCL)$, that is the same as our result.

The pentoxifylline-enhanced VCL was probably the result of the increases in ALH that we also observed. Amplitude of lateral head displacement has previously been correlated with the efficiency of cervical mucus penetration.⁽²⁰⁾ In addition, Bongso et al noted a higher ALH in sperm of fertile men than in those of an infertile group.⁽²⁾ Increased ALH may improve VCL by moving the sperm head further from side to side, although increased beat cross frequency may complement this action by making those movements faster. An increase in ALH and VCL may reflect an increase in energy consumption facilitated by pentoxifylline or a decrease in the energy required to deflect the sperm head because pentoxifylline had also been shown to decrease membrane rigidity.⁽¹³⁾ The sum of these effects may make the penetrating power of the sperm greater, thus improving fertilization rates.

Our results have shown that, during exposure to pentoxifylline, spermatozoa maintain the accelerated movement pattern for several hours. The duration of the pentoxifylline effects was shorter than 24 hours.

There are differences in basic protocol of each reports e.g. type of sperm preparation, method of sperm motility assessment, pentoxifylline concentration. Therefore, it is difficult to compare the results to other reports. In this study, the pentoxifylline action was combined with Percoll gradient. The resulting improvement of motility was thus attributable to a combination of stimulation and selection. Pentoxifylline was used at a concentration of 3.6 mM which has previously been shown to increase in vitro sperm motility and fertilization rates.⁽¹⁵⁾ Sperm motility characteristics in our study was assessed using a Hamilton-Thorn motility analyser, which has been shown to allow objective quantitative analysis.⁽²¹⁾

The present study shows interest in the use of pentoxifylline as a sperm movement enhancer for normozoospermic semen. It shows a beneficial effect in some sperm movement parameters. At the moment it seems that more basic and clinical research is necessary before pentoxifylline or other methylxanthines are considered as a standard therapy in fertility clinics. Additionally, more investigation on the impact on sperm ultrastructure and possible mutagenic effects of pentoxifylline or its metabolites is obligatory.

In conclusion, we have shown that pentoxifylline increases the quality of sperm motility in normal men. However, whether this change leads to an increase in fertilizing ability requires further study.

References

1. Mahadevan MM, Trouson AO. The influence of seminal characteristics on the success rate of human in vitro fertilization. *Fertil Steril* 1984 Sep; 42(3) : 400-5

2. Bongso TA, Ng SC, Mok H, Lim MN, Teo HL, Wong PC, Ratnam SS. Effect of sperm motility on human in vitro fertilization. *Arch Androl* 1989; 22(3) : 185-90
3. Schoenfeld C, Amelar RD, Dubin L. Stimulation of ejaculated human spermatozoa by caffeine. *Fertil Steril* 1975 Feb; 26(2) : 158-61
4. Aitken RJ, Best FSM, Richardson DW, Schats R, Simm G. Influence of caffeine of movement characteristics, fertilizing capacity and ability to penetrate cervical mucus of human spermatozoa. *J Reprod Fertil* 1983 Jan; 67(1) : 19-27
5. Hammitt DG, Bedia E, Rogers PR, Syrop CH, Donovan JF, Williamson RA. Comparison of motility stimulants for cryopreserved human semen. *Fertil Steril* 1989 Sep; 52(3) : 495-502
6. Loughlin KR, Agarwal A. Use of theophylline to enhance sperm function. *Arch Androl* 1992 Mar-Apr; 28(2) : 99-103
7. Aparicio NJ, de Turner EA, Schwarzstein L, Turner D. Effect of the phosphodiesterase inhibitor pentoxifylline on human sperm motility. *Andrologia* 1980 Jan-Feb; 12(1) : 49-54
8. Garbers DL, Lust WD, First NL, Lardy HA. Effects of phosphodiesterase inhibitors and cyclic nucleotides on sperm respiration and motility. *Biochemistry* 1971 May; 10 : 1825-31
9. Tash JS, Means AR. Cyclic adenosine 3',5' monophosphate, calcium and protein phosphorylation in flagellar motility. *Biol Reprod* 1983 Feb; 28(1) : 75-104
10. Sikka SC, Hellstrom WJG. Functional evaluation and motility parameters of pentoxifylline-stimulated cryopreserved human sperm. *Assis Repord Technol/Androl* 1991; 1 : 309-19
11. World Health Organization. WHO Laboratory Manual for the Examination of Human Semen and Semen-Cervical Mucus Interaction. 3rd edn. Cambridge : The Press Syndicate of the University of Cambridge, Cambridge, 1993.
12. Mortimer D, Serres C, Mortimer ST, Jouannet P. Influence of image sampling frequency on the perceived movement characteristics of progressively motile human spermatozoa. *Gemete Res* 1988 Jul; 20(3) : 313-27
13. Ward A, Clissold SP. Pentoxifylline : a review of its pharmacodynamic and pharmacokinetic properties, and its therapeutic efficiency. *Drugs* 1987 Jul; 34(1) : 50-60
14. Perreault SD, Rogers BJ. Relationship between fertilizing ability and c AMP in human spermatozoa. *J Androl* 1982; 3 : 396-401
15. Yovich JM, Edirisinghe WR, Cummins JM, Yovich JL. Preliminary results using pentoxifylline in a pronuclear stage tubal transfer (PROST) program for severe male factor infertility. *Fertil Steril* 1988 Jul; 50(1) : 179-81
16. Rees JM, Ford WCL, Hull MGR. Effect of caffeine and of pentoxifylline on the motility of human spermatozoa. *J Reprod Fertil* 1990 Sep; 90(1) : 147-56
17. Sikka SC, Hellstrom WJG. The application of pentoxifylline in the stimulation on sperm motion in men undergoing electroejaculation. *J Androl* 1991 May-Jun; 12(3) : 165-70
18. Barlow P, Delvigne A, Van Dromme J, Van Hoeck J, Vandenbosch K, Leroy F. Predictive value of classical and automated sperm analysis for in vitro fertilization. *Hum Reprod* 1991 Sep; 6(8) : 1119-24
19. Lewis SEM, Moohan JM, Thompson W. Effects of pentoxifylline on human sperm motility in normospermic individuals using computer-assisted analysis. *Fertil Steril* 1993 Feb; 59(2) : 418-23
20. Aitken RJ, Warner PE, Reid C. Factors influencing the success of sperm-cervical mucus interaction in patients exhibiting unexplained infertility. *J Androl* 1986 Jan-Feb; 7(1) : 3-10
21. Olds-Clark P, Baer HM, Gerber WL. Human sperm motion analysis by automated (Hamilton-Thorn Motility Analyser) and manual (Image-80) digitization systems. *J Androl* 1990 Jan-Feb; 11(1) : 52-8