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นิพนธ์ต้นฉบับ

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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นเรศร สุขเจริญ, อุษณีย์ เจตน์สว่าง, เย็นจิต จันทร์ประสิทธิ์, จิราพร เหงี่ยมวิจาวัฒน์, 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 24ชั่วโมง 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% CO_2 in air) for different periods of time before movement analysis. The samples of each group were annlysed 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 consider4d 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 exposue to pentoxifylline for 24 hours, the sperm movement characteristics did not differ from corresponding controls.

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

Sperm motlity 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 phophodiesterase 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 aceleration, 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 studies. 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 charateristics 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 increased 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 B arlow,(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 mem brane 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 fertilzation rates.(15) Sperm motility characteristics in our study was assessed using a Hamiltion-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 in obligatory.

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

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