THE EFFECT OF PENTOXIFYLLINE ON THE SPERM MOTILITY IN VITRO

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ABSTRACT **OBJECTIVE**: To study the effect of pentoxifylline(PTF) on the sperm motility of asthenozoospermic patients and to explore the mechanism to improve the sperm motility. **METHODS**: We used CASA system to measure the parameters of the sperm motility which was treated with PTF in vitro compared with the untreated controls. **RESULTS**: The addition of PTF significantly increased the percentage of forwardly progressive spermatozoa(rate a+ b) ($P < 0.01$), especially the percentage of fast forwardly progressive spermatozoa (rate a) ($P < 0.005$); The improvement of the percentage of non-motile spermatozoa was not remarkable(rate d)($P > 0.05$); The increased rate of sperm motility of poor-motile spermatozoa was more obviously than that of well-motile spermatozoa($P < 0.05$). **CONCLUSION**: PTF can improve the sperm motiltiy of asthenozoospermic patients effectively.

KEY WORDS Pentoxifylline; Asthenozoospermia; The sperm motility

己酮可可碱对体外精子活力的影响

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摘要 目的: 研究己酮可可碱(Pentoxifyline, PTF)对弱精患者精子活力的影响, 探讨 PTF 提高精子活力的作用机理。方法: 采用计算机辅助精子分析(CASA)系统, 以同一份精液标本为自身对照, 测定 PTF 处理前后精子活力参数值。结果: PTF 处理后精子活力(a+ b 级)显著提高($P < 0.01$), 对提高快速前向运动精子活力(a 级)有极显著意义($P < 0.005$), 而对精子活动率的提高不显著($P > 0.05$); PTF 对活动力较好精子活力的提高不及活动力差的明显($P < 0.05$)。结论: PTF 能有效提高弱精患者精子活力。

关键词 己酮可可碱; 弱精; 精子活力

Oligozoospermia, asthenozoospermia and oligoasthenozoospermia are considered to cause about 46% of male - factor infertility[1]. Oligozoospermia means small amounts of spermatozoa in semen, and asthenozoospermia refers to low activity of spermatozoa. If small amounts and low activity are both exist, we call it oligoasthenozoospermia. Until now few effective drugs has been used to treat these diseases, only HCG has been used to increase the number of spermatozoa. Now it is urgent to develop a drug that can improve sperm motility. The use of pentoxifylline (PTF) as a sperm motility stimulant has already been reported in foreign literatures, but little has been done in our country. In this research semen samples were obtained from 19 patients considered to have asthenozoospermia. After incubation with PTF in vitro, the parameters of sperm motility were measured. Then we summarized and explored the mechanism of PTF in improving the sperm motility.

1 Material

1.1 Semen collection: Semen samples were obtained by masturbation or automatic massage from 19 patients in obstetrics & gynecology infertility clinic in Sir Run Run Hospital from May 2000 to Aug. 2000. All the patients were aged 25-35 and kept sexual abstinence for 3-5 days. Inlet standard: asthenozoospermia, according to WHO standard, sperm motility (rate a+ b) < 50%, (rate a) < 25%.

1.2 Drugs and Reagents: TRENTAL (contain PTF 400mg/tablet; Batch No. 19971240B539), Hoechst Marion Roussel, German; Human Tube Fluid(HTF; Batch No. 996200776), Owen Company, USA.

1.3 Instruments: Computer Aid Sperm Analysis (CASA) system, Hangzhou Huali Medical Instruments INC.

2 Method

2.1 The CASA system is one of the most common systems used in automatic sperm analysis. As the percentage of forwardly progressive spermatozoa (rate a+b) and fast forwardly progressive spermatozoa (rate a) can reflect the sperm motility and conception ability, they were selected to be analyzed by statistical method. The results are accurate and reliable.

2.2 Procedure: The PTF tablets were crashed into pieces and dissolved in HTF to final concentration of 4.0 mmol/l. The freshly collected semen specimens were liquefied after 0.5 hr at routine temperature and then divided into two aliquots. One aliquot was mixed with double-volume PTF solution and incubated at 37℃ for lhr. Another with no addition served as control. The motility parameters were determined by CASA system respectively.

3 Result

The following table shows the motility values of semen specimens before and after PTF treatment, t-test was performed.

Comparison of sperm motility between PTF-treated group and untreated control group ($\bar{x} \pm s$, n=19)

groups	Percentage of forwardly progressive spermatozoa (a+b)	Percentage of fast forwardly progressive spermatozoa (a)	Percentage of non-motile spermatozoa (d)
PTF-treated group	21.32±10.65	8.42±5.54	60.56±13.53
Untreated controll group	33.47±11.65	18.79±7.12	55.10±13.36

The percentage of forwardly progressive spermatozoa (rate a+b) was significantly increased after PTF treatment ($P<0.01$), especially that of fast forwardly progressive spermatozoa (rate a) ($P<0.005$). The difference in the percentage of non-motile spermatozoa (rate d) between the two groups was not significant ($P>0.05$).

According to the sperm motility, all cases were divided into extremely-asthenozoospermic group (a < 10%) and general-asthenozoospermic group (10% ≤ a < 25%). After the sperm was treated with PTF, the increased rates of its motility in both groups were compared by rank sum method. The result revealed that the increased rate of the sperm motility in extremely-asthenozoospermic group was markedly higher than that in general-asthenozoospermic group ($P<0.01$), which suggested that PTF executed a more significant effect on poor-motile spermatozoa than well - motile spermatozoa.

4 Discussion

PTF, a derivative of methylxanthine, can improve the sperm motility, induce the acrosome reaction, enhance the sperm ability of binding the pellucid zone and increase the rates of fertilization in vitro. PTF can inhibit the activity of phosphodiesterase, increase intracellular cAMP concentration. By activating a cAMP-dependent protein kinase, cAMP then induces the phosphorylation of the specific protein related to the movement of sperm tail[2]. Protein phosphorylation appears to be a necessary step in the intracellular signal pathway that initiates the activation of sperm motility[3]. In 1994 Wang etc. investigated the role of PTF in sperm motility and cAMP concentration after the sperm was frozen and thawed. The result showed the sperm motility was increased obviously in initially lhr while the cAMP concentration was 3-fold increased compared with the controls[4]. It was proved that the mechanisms was that PTF-induced inhibition of the activity of phosphodiesterase reduced cAMP degradation and then increased cAMP concentration and improved sperm motility. In 1999 the research of the PTF-stimulated action on intracellular signal molecules in hamster sperm suggested that: (1) the PTF-stimulated early onset of sperm capacitation may be mediated by an early rise in cAMP and [Ca2+] and involves protein kinase A activity, (2) PTF-stimulated acrosome reaction may require phospholipase A2 and protein kinase C activity[5]. Wang Ru-yao etc., scholars in Shanghai second medical university, discussed the mechanism of PTF in sperm energy metabolism involving the function of mitochondria and its oxidative phosphorylation. It was believed that besides stimulating the sperm motility PTF can enhance the function of sperm mitochondria and its oxidative phosphorylation.

Successful fertilization and its potency depend to a great extent on the quality of forwardly progressive spermatozoa, so its percentage has important clinical significance. This research revealed that PTF has marked improvement on the percentage of forwardly progressive spermatozoa, especially that of fast forwardly progressive spermatozoa. In this study, there were 19 cases with asthenozoospermia, of which 7 cases obtained a sperm motility of rate a≥25 and 3 cases obtained a sperm motility of rate (a+b) ≥50 after being treated with PTF, which reached normal level. Although the absolute effective rate wasn't high, it played a practical role in IVF and provided beneficial help for selecting optimal sperm in the field of assisted reproduction technology. This experimental result also indicated that PTF executed a more significant effect on poor-motile spermatozoa than well-motile spermatozoa, which undoubtedly will bring a Gospel to the patients with extremely-asthenozoospermia.

In 1996 Negri P etc. performed a controlled trial on the effectiveness of PTF for intrauterine insemination[6]. The result showed a significant difference in pregnancy rate between PTF treated group and controlled group. Abortions and malformations equally distributed in two groups. Such properties indicate that PTF may be a useful tool for improving sperm motility and in vitro fertilization rates.

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