OBJECTIVES: To evaluate the in vitro effect of Ritalinic acid on human sperm parameters and oxidative stress.

Design: An in vitro concentration and time course study.

Materials and Methods: Sperm samples (n=13) from healthy donors were analyzed after a single semen analysis according to WHO (2010). Density gradient centrifugation was performed to isolate motile sperm. The following tests were performed manually: sperm viability and motility as assessed by computer aided semen analysis (CASA); oxidative stress (ORP) was measured using the MiOXSYS analyzer; sperm viability was determined with the vital stain Eosin Y. The ORP was measured in sperm suspension by using the MiOXSYS analyzer (Aytu Bioscience, Eaglewood, CO) and was presented as mV/10^6 sperm/mL. To assess the effects of time on ORP measures, samples were incubated in the MiOXSYS analyzer; measurements begin automatically. ORP is measured in milli Volts (mV).

Conclusions: Ritalinic acid increases sperm motility at any concentration tested. Even the lowest concentration (1 ng/mL) which is significantly lower than the reported blood plasma level (150 minutes). Sperm vitality and motility were assessed manually and by computer assisted semen analysis (CASA). In addition, the weight of the testes and seminal vesicles decreased. MPH was reported to reduce oxidative stress.

Ritalinic acid (RA), the major inactive metabolite of MPH, is one of the isomers of amphetamine. It is a sympathomimetic drug that acts on presynaptic dopamine transporter. The mechanism of action includes inhibition of the re-uptake of norepinephrine and dopamine into the presynaptic neuron or an increase of their intrasynaptic concentrations. RA was used in this study to test the effect of MPH or its metabolite(s) on sperm parameters such as acrosome reaction, DNA fragmentation or membrane integrity.

Ritalinic acid on human semen parameters.

Introduction

Attention-deficit hyperactivity disorder (ADHD) is a frequent neurodevelopmental disorder with an increasing prevalence over the last 2 decades. The most commonly used first line pharmacologic treatment for ADHD is methylphenidate (MPH). The mechanism of action of MPH in ADHD is still a matter of debate, but several studies indicated that MPH inhibits the presynaptic dopamine transporter. The mechanism of action includes inhibition of the re-uptake of norepinephrine and dopamine into the presynaptic neuron or an increase of their intrasynaptic concentrations by increasing their release into the extraneuronal space. MPH is given in the normal dosage for clinical use, the plasma concentration of Ritalinic acid reaches a maximal concentration of 1 ng/ml within 2.5 hours. Ritalinic acid is soluble in water and due to its small size, readily passes the blood brain and testicular barriers. The circulating concentrations of RA greatly exceeded that of the parent drug. If MPH is given in the normal dosage for clinical use, the plasma concentration of Ritalinic acid reaches a maximal concentration of 1 ng/ml within 2.5 hours. Ritalinic acid is soluble in water and due to its small size, readily passes the blood brain and testicular barriers. The circulating concentrations of RA greatly exceeded that of the parent drug. If MPH is given in the normal dosage for clinical use, the plasma concentration of Ritalinic acid reaches a maximal concentration of 1 ng/ml within 2.5 hours. Ritalinic acid is soluble in water and due to its small size, readily crosses the blood brain and testicular barriers.

Conclusions:

1. Motility: All concentrations of RA used in this study significantly (P<0.05) increased sperm motility with a maximum after 240 minutes of incubation (Figure 1). This increase was maintained with increasing concentrations of up to 100 ng/ml. Thereafter, a decrease was observed after 300 minutes of incubation, treated samples, even though motility remained significantly higher compared to control.

2. Viability: Addition of RA to the sperm samples caused sperm to retain their vitality (Figure 3).

3. ORP: An anticipated, but non-significant (P=0.3125 and P=0.1953) increase in ORP levels compared to the control was observed in the control group after 240 and 300 minutes of incubation, respectively (Figure 4).

Materials and Methods

Following the approval of the study by the Institutional Review Board of the Cleveland Clinic, a total of 13 semen samples were collected from healthy donors by masturbation after 2-3 days of abstinence. All samples were allowed to liquefy completely for 20 minutes at 37°C before further processing. The initial semen analysis was performed according to the WHO 5th edition (2010). Density gradient centrifugation was performed to separate motile sperm. Given the achieved plasma concentration of 1 ng/ml during treatment with Ritalinic acid, each sample was incubated for 5, 10, 30, 60, and 120 minutes with different concentrations (1, 10, and 100 ng/ml) of Ritalinic acid, the major metabolite of methylphenidate, and compared to a control. Incubation was based on the half-life of Ritalinic acid in plasma (150 minutes). Sperm vitality and motility were assessed manually and by computer assisted semen analysis (CASA). In addition, the weight of the testes and seminal vesicles decreased. MPH was reported to reduce oxidative stress.

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