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COMPUTER-ASSISTED SEMEN ANALYSIS OF RAT SPERMATOZOA AFTER AN INTRAPERITONEAL ADMINISTRATION OF INSECTICIDE DIAZINON

ANALIZA COMPUTERIZATĂ A MATERIALULUI SEMINAL SUB EFECTUL ADMINISTRĂRII INTRAPERITONEALE DE INSECTICIDE PE BAZĂ DE DIAZINON LA ŞOBOLAN

TOMAN, R., HLUCHÝ, S., MASSÁNYI, P., ŠIŠKA, B., LUKÁČ, N., SLIVKOVÁ, J., GOLIAN, J., ČUPKA, P.

Slovak Agricultural University in Nitra, Slovak Republic

The aim of this study was to reveal the effect of diazinon on the rat spermatozoa motility characteristics using the computer-assisted semen analysis (CASA). Motility, progressive motility, DAP, DCL, DSL, VAP, VCL, VSL, STR, LIN, WOB, ALH, and BCF after the diazinon i.p. administration of 20 mg/kg b.w. were evaluated. 36 hours after the diazinon administration, only slight decrease in VCL, DCL and increase in percentage of progressive motility in the diazinon-treated group. Significant decrease (P<0.01) was only observed in BCF in diazinon-treated group. Computer-assisted semen analysis (CASA) of rat sperm motility showed that acute diazinon administration slightly affected the rat sperm motility which can be the first step in the decreased fertilization capacity caused by pesticides. Further investigation of reproductive effects of diazinon is needed.
Key words: diazinon, CASA, rat, spermatozoa, motility

Introduction

Diazinon [*O,O*-Diethyl *O*-(2-isopropyl 6-methyl 4-pyrimidinyl phosphorothioate] is a nonsystemic organophosphate insecticide used to control pest insects in soil, on ornamental plants, and on fruit and vegetable field crops. It has also veterinary uses against fleas and ticks. In the environment, diazinon is quickly broken down into a variety of other chemicals (ATSDR, 2006). Toxicity of diazinon is realized through the inhibition of enzyme acetylcholinesterase which biological role is the termination of impulse transmissions at cholinergic synapses within the nervous system by rapid hydrolysis of the neurotransmitter, acetylcholine (Schumacher et al., 1986). Except of the neurological effects of diazinon, some authors have described the reproductive effects in mammals. Pina-Guzmán et al. (2005) revealed the high sensitivity of spermatozoa exposed during the late steps of maturation to diazinon. Sperm motility, viability, and morphology showed significant alterations 8 days after the treatment. Structural changes of the

testis of bluegill, *Lepomis macrochirus*, have also been described after the diazinon exposure (Dutta and Meijer, 2003).

The aim of the study was to find out whether diazinon has a significant effect on the rat sperm motility parameters.

Materials and Methods

Nineteen males Wistar rats were divided to two groups, diazinon-treated group (B) and control group (A), each containing 9 males. The males were housed individually in plastic cages. The animals had unlimited access to drinking water and feed. Sexually mature male rats were administered a single intraperitoneal dose (20 mg/kg b.w.) of diazinon (Sigma-Aldrich, USA, purity 99%). The testis and epididymis were sampled 36 hours after the diazinon administration. Spermatozoa obtained from the cauda epididymis were subsequently diluted with tempered (37°C) physiological solution (20 µl). The sample was located into the Makler chamber (Sefi-Medical Instruments, Germany). Analysis was realized using a CASA system – SpermVision (Minitüb, Tiefenbach, Germany) with Olympus BX 51 (Olympus, Japan) microscope. In the samples, % of motile spermatozoa, % of progressive motility, DAP (distance average path, µm), DCL (distance curved line, µm), DSL (distance straight line, µm), VAP (velocity average path, µm/s), VCL (velocity curved line, µm/s), VSL (velocity straight line, µm/s), STR (straightness, VSL/VAP, %), LIN (linearity, VSL/VCL, %), WOB (wobble, VAP/VCL, %), ALH (amplitude of lateral head displacement, µm), and BCF (beat cross frequency, Hz) were evaluated. Means, ±S.D. and t-test were conducted for the statistical analysis of differences between the control and experimental groups.

Results and Discussions

The data from CASA analysis are given in Table 1.

When the two groups were compared using CASA, it was found that the average values for motility, DAP, DSL, VAP, VSL, STR, LIN, WOB, and ALH were almost the same. Percentage of progressive motility increased insignificantly in the diazinon-treated group from 14.17 to 18.99%. On the other hand, DCL slightly decreased in the experimental group. VCL decrease (83.81 to 71.92 µm/s in control and experimental group, respectively) was more evident but still statistically insignificant. Beat cross frequency significantly decreased ($P < 0.01$) in the diazinon-exposed group.

Table 1.

Semen parameters measured with CASA

Parameters	Group A	Group B
	Mean ± SD	*Mean ± SD*
Motility (%)	31.46 ± 15.47	30.96 ± 17.30
Progressive motility (%)	14.17 ± 11.05	18.99 ± 11.84
DAP (µm)	20.37 ± 4.66	20.28 ± 6.93
DCL (µm)	33.42 ± 7.25	28.35 ± 11.35
DSL (µm)	15.73 ± 3.19	15.94 ± 6.44
VAP (µm/s)	51.77 ± 11.70	51.67 ± 24.08
VCL (µm/s)	83.81 ± 17.94	71.92 ± 24.49
VSL (µm/s)	39.97 ± 7.89	40.07 ± 16.13
STR (%)	0.74 ± 0.08	0.70 ± 0.16
LIN (%)	0.47 ± 0.05	0.47 ± 0.11
WOB (%)	0.59 ± 0.06	0.57 ± 0.12
ALH (µm)	5.40 ± 1.06	5.71 ± 2.03
BCF (Hz)	17.05 ± 2.24	12.01 ± 4.52 **

** $P < 0.01$; DAP - distance average path, DCL - distance curved line, DSL - distance straight line, VAP - velocity average path, VCL - velocity curved line, VSL - velocity straight line, STR – straightness, LIN - linearity, WOB - wobble, ALH - amplitude of lateral head displacement, BCF - beat cross frequency

The evaluation of spermatozoa is an important factor that must be accurately analyzed to reveal the fertility disorders caused i.e. by environmental pollutants like pesticides. In the present work, the computer assisted semen analysis has been used for identification of changes in the spermatozoa movement which can be a reason of the fertility decrease or failure. There has been a little evidence of the reproductive toxicity of diazinon. However, Swan et al. (2003) have noted that diazinon is one of the most important insecticides along with herbicides like alachlor and atrazine that is associated with decreased semen quality in men. Structural changes in the bluegill testis were also described. The changes in the seminiferous tubule diameter, germ cells diameter and connective tissue have been found depending on the time of exposure. The reduction in the tubule, tubule lumen and spermatozoa diameter were highly significant after 2 weeks of exposure to diazinon (Dutta and Meijer, 2003). In spermatozoa, an alteration in sperm chromatin condensation and DNA damage during late spermatid differentiation has been revealed after the diazinon injection to mice (Pina-Guzmán et al., 2005). The main toxic action of diazinon is related to phosphorylation of proteins. Chemical alterations in sperm nuclear proteins, which pack DNA during the last steps of spermatogenesis, contribute to male reproductive toxicity. Sánchez-Pena et al. (2004) have also described the alteration of sperm chromatin condensation which could be reflected in an increased number of cells more susceptible to DNA denaturation. Human sperm chromatin is a sensitive target to organophosphorous

insecticide exposure. The data collected by Pina-Guzmán et al. (2005) suggest that spermatozoa exposed during late steps of maturation were the targets of diazinon exposure. Thus, toxicant-induced DNA damage in this repair-deficient period of late spermatogenesis and epididymal sperm maturation would not be repaired and may be manifest as increased DNA fragmentation (Rubes et al., 2005).

When measuring sperm velocity, CASA evaluates three main motility characteristics; velocity average path (VAP), velocity straight line (VSL), and curvilinear velocity (VCL). In our experiment, there were no significant differences in these parameters between the groups. Decrease in VCL, even insignificant, could suggest some impairment of the motion ability as curvilinear velocity is the total distance in an observation period that the sperm head can traverse, and it has the largest numerical value of the three velocities. Anyway, VSL measuring the straight-line distance in an observation period remains unaffected.

Another useful measurement is the beat cross frequency (BCF), which counts the number of times that the sperm head crosses the direction of movement. Beat cross frequency is a valuable measurement because it assesses the number of times the flagellar beat changes its pattern. The spermatozoa from the diazinon-exposed group had significantly smaller BCF value than that of the controls. Similarly, Lifeng et al. (2006) have found significant decrease in BCF after the exposure to insecticide fenvalerate among occupational workers. Golas et al. (2004) revealed that the BCF trait depends on the genetic factors located in chromosome 7q11. This chromosome probably controls BCF in mice and this region may contribute to gamete production.

Conclusions

The data show the slight acute effect of diazinon administration on the epididymal spermatozoa of rats. An insignificant decrease in motility characteristics (VCL and DCL) and significant decrease in frequency parameter (BCF) indicate the possibility of adverse effect of diazinon on male fertility. Another study on the diazinon effects covering the histopathology and hormonal regulation of the testis and epididymis should be provided to reveal the mechanism of these effects on the spermatozoa development and function.

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