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The effect of R-(-)-deprenyl administration on reproductive parameters of rat males

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ABSTRACT

The aim of the study was to investigate the effect of R-(-)-deprenyl administration on the reproductive parameters of rat males. After 30 days of intraperitoneal administration of saline or 0.0025 mg/kg (10^{-5} mol/l) of R-(-)-deprenyl dissolved in saline, males were mated with females of the same strain. Subsequently, animals were killed by thiopental, and their blood and sperm were collected. We found that epididymis of males exposed to R-(-)-deprenyl had higher sperm count ($P<0.05$), and females who mated with these males gave birth to a greater number of offspring ($P<0.05$) compared to control. The blood of experimental animals contained higher levels of testosterone ($P<0.05$), FSH ($P<0.01$), and total antioxidants ($P<0.01$). We did not detect sperm DNA fragmentation in control or in experimental males. Interestingly, round spermatids were often observed inside seminiferous tubules of experimental animals, but obviously without any negative consequences on male fertility. Our findings could be verified on a sample of human male volunteers treated for infertility, because human organism tolerate higher doses of R-(-)-deprenyl, which is a selective inhibitor of monoamine oxidase B employed in our experiment and used in the therapy of Parkinson's disease, rather well.

1. Introduction

A number of studies conducted over the past decades confirmed that sperm counts in males from western industrialized countries are on the decline (Carlsen et al., 1992; Rolland et al., 2013; Swan et al., 1997). Moreover, the semen contains a greater proportion of deformed spermatozoa (Le Moal et al., 2014). Since these changes are recent and appear to be occurring internationally, it has been presumed that they reflect the adverse effects of environmental or lifestyle factors on males rather than genetic changes (Adams et al., 2014; Joo et al., 2012). Many studies have supported the view that potential environmental endocrine disrupters (estrogen-like chemicals, plastics, therapeutic drugs, and other industrial chemicals) should be blamed for such situation (Dindyal, 2004; Manfo et al., 2014).

Monoamine oxidases (MAO) are enzymes that degrade biogenic monoamines. Two MAO subtypes, MAO-A and MAO-B, have different inhibitor and substrate specificities. Both forms are bound to the outer mitochondrial membrane. MAO-A has a higher affinity for the serotonin and noradrenaline substrates, and MAO-B has a higher affinity for β-phenylethylamine. Dopamine is a common substrate of both MAO. MAOs are involved in many behavioral processes and their inhibition. Thus, using MAO inhibitors for therapeutic or experimental reasons has marked effects on brain function, blood pressure regulation, and detoxification of potentially harmful exogenous amines (Singer and Ramsay, 1995).

The lack of information on whether long-term MAO inhibitor administration can influence the course of spermatogenesis and ability to fertilize females prompted us to investigate this area. We began our work by employing the potent MAO-B inhibitor R-(-)-deprenyl, which is known for its neuroprotective effects (Semkova et al., 1996), its antiapoptotic effects in both cultured neurons (Le et al., 1997) and animal models (Giladi et al., 2001), and the reduction of oxidative radical production (Ebadi et al., 2002). In human medicine, the R-(-)-deprenyl (selegiline) is used mainly in the treatment of Parkinson's disease. It can be used on its own or in a combination with another agent, most often L-DOPA (levodopa), because of its ability to increase striatal dopaminergic tone through inhibition of MAO-B (Riederer et al., 2004). Patients maintained on R-(-)-deprenyl needed L-DOPA later compare to their placebo-treated peers, and those who were on

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levodopa plus R-(-)-deprenyl lived considerably longer compared to patients on levodopa alone (Knoll, 2000).

In our preliminary experiment, we tested the effect of two R-(-)-deprenyl doses on the central nervous system (Danková et al., 2014) and sperm count of rat males (data not published yet). We found that administration 10^{-3} mol/l of R-(-)-deprenyl significantly increased the number of glial fibrillary acidic protein (GFAP) positive astrocytes in spinal cord. The sperm count in these males was slightly higher, but did not differ from controls. On the other hand, the number of GFAP positive astrocytes in males exposed to 10^{-5} mol/l of R-(-)-deprenyl did not differ from control, although the sperm count increased significantly. Based on these results, we decided to continue with our experiments, administering the R-(-)-deprenyl dose of 10^{-5} mol/l. This dose is low enough not to influence the central nervous system but high enough to increase the sperm count.

2. Materials and methods

2.1. Animals

All procedures involving animals adhered to the guidelines of the Committee for Ethical Control of Animal Experiments at Šafárik University and the Slovak State Veterinary and Alimentary Administration (permission no. Ro-1757/10-221b). All efforts were made to minimize both the number of animals and their suffering.

Male Wistar rats (390 g, 85–90 days old) were obtained from the animal facility (Laboratory of Research Biomodels) of the Šafárik University. The animals were given free access to standard diet and water and were exposed to a 12 h light/12 h dark cycle. Animals were randomly divided into two groups: control (C) and experimental (D). Males were injected intraperitoneally with saline (C) or with the 0.0025 mg/kg/day (10^{-5} mol/l) dose of R-(-)-deprenyl (M003, Sigma, St. Louis, USA) dissolved in saline (D) daily for 30 days. After the last drug administration, each male was caged for the next week with young virgin female of the same strain. Females were allowed to deliver, and the number of youngsters was recorded.

2.2. Sperm collection and concentration

After one week of cohabiting with females, males were killed by a lethal dose of thiopental (40 mg/kg; ICN Czech Pharma, Prague) administered in the morning between 08:00–09:00, and their blood was collected by cardiac puncture to detect hormone levels and total antioxidant status (see below). Tails of both epididymis were immediately removed and washed twice in medium 199 with Hank's salts (12350-039, Gibco, Carlsbad, USA) to eliminate as much blood and fat from the surface as possible. Finally, both tails from one animal were placed in 1 ml of the same medium. Sterile razors were used to do several sections on the surface of each epididymis and spermatozoa were allowed to flow out into drop. Drops were mixed well by gentle aspirating the sample 10 times into disposable plastic pipette.

Sperm concentration was estimated by following the manual for examination and processing of human semen (WHO, 2010). Briefly, fixative solution was prepared by dissolving 50 g of NaHCO3 and 10 ml of 35% (v/v) formalin in 1000 ml of purified water. Subsequently, 50 μl of sperm was diluted with 950 μl of fixative solution (dilution 1:20). Then, 10 μl of diluted spermatozoa was loaded into both ends of improved Neubauer haemocytometer and sperm were allowed to settle in a humid chamber. The samples were assessed within 10–15 min. The results are given as the number of spermatozoa $\times 10^6$/ml.

2.3. DNA fragmentation of spermatozoa

Sperm (1×10^6) isolated from epididymis was washed twice with PBS without calcium and magnesium. The lysis of sperm was performed in lysing buffer containing 10 mM EDTA, 0,5% Triton X-100, and 10 mM TRIS (pH 8,0). Proteinase K (1 mg/ml) was added and sperm was incubated for 1 h at 37 °C followed by 10-min. incubation at 70 °C. RNAase (200 μg/ml) was then added and cells were incubated for another 1 h at 37 °C. Samples were transferred to 2% agarose gel and run at 40 V for 3 h followed by UV illuminator visualization. All chemicals were purchased from Sigma.

2.4. Levels of hormones and total antioxidant status (TAS)

Cardiac puncture was used to collect 3–4 ml of whole blood, which was delivered to the laboratory immediately. The hormone parameters were determined in blood serum using the automatic analyzer Dynex DS2 (DYNEX Technologies, Chantilly, USA) according to the ELISA kits manufacturer's instructions. The levels of LH and FSH were measured by direct ELISA using the commercial kits from Uscn Life Science Inc. (CEA441Ra and CEA830Ra, Houston, USA) while the levels of testosterone were measured using the commercial kit from DEMEDITEC (DEV9911, Diagnostics GmbH, Kiel-Wellsee, Germany). The results of LH are given as pg/ml ± S.D., the results of testosterone are given as ng/ml ± S.D., and the results of FSH are given as IU/l ± S.D.

The TAS parameters in blood serum have been determined using the automatic analyzer (COBAS MIRA, Roche, Switzerland), employing commercial TAS kit (NX2332 RANDOX, United Kingdom). The levels of TAS are expressed as mmol/l ± S.D.

2.5. Histological examination

Left testicles from each animal served to detect antioxidant enzymes superoxide dismutases and catalase (data not shown here, work in progress). Right testicles were used for histological examination of semi-thin sections. Briefly, the 1 mm^3 excisions from control and experimental animals were fixed by immersion in 3% glutaraldehyde and postfixed in 1% osmium tetroxide (both in 0.15 M cacodylate buffer, pH 7.2–7.4). After dehydration in acetone, they were transferred to propylene oxide and embedded in Durcupan ACM (Fluka). Semi-thin 1 μm sections of specimens were cut on the ultramicrotome LKB Nova using glass knives. The sections were stained with toluidine blue and examined under a light microscope Axio Lab A1.

2.6. Statistical analysis

The results were analyzed by two-tailed t-test where $P < 0.05$ was considered statistically significant.

3. Results

3.1. Sperm concentration and number of youngsters

The results are shown in Table 1. We found that R-(-)-deprenyl administration significantly increased sperm concentration in experimental group. Sperm count in males after the exposure to R-(-)-deprenyl increased by more than 19% compared to control ($P < 0.05$). Moreover, females who mated with experimental males delivered more youngsters compared to control animals ($P < 0.05$).

3.2. Sperm DNA fragmentation

Electrophoresis did not reveal any differences in DNA fragmentation between the sperm from experimental and control animals (Fig. 1).