Study of high deprenyl dose on the preimplantation embryo development and lymphocyte DNA in rat

J. Mihalik[1], P. Kravčuková[2], T. Špakovská[1], M. Mareková[3] and K. Schmidtová[1]

[1] *Department of Anatomy, Medical Faculty, P. J. Šafárik University, Šrobárova 2, 040 01 Košice, Slovakia*
[2] *Neurobiological Institute of the Slovak Academy of Sciences, Šoltésovej 4, 040 01 Košice, Slovakia*
[3] *Department of Medical Chemistry and Biochemistry, Medical Faculty, P. J. Šafárik University, Tr. SNP 1, 040 01 Košice, Slovakia*

Abstract. To investigate the role of potent MAO-B inhibitor deprenyl in fertilized females, we have evaluated the effect of chronic treatment with deprenyl at a high dosage on preimplantation embryo development and DNA damage in blood lymphocytes in Wistar rats. We have found that the number of embryos isolated from both uterus and oviduct per rat was significantly lower in the experimental group. Almost 14% of embryos in experimental animals were flushed from oviducts compared to 1.95% of those in the control rats. Morphological analysis of embryos isolated from deprenyl-treated animals had revealed impaired rates in the distribution pattern compared with controls. But deprenyl administration had no significant effect on the mean number of cells in morulae or even blastocysts. On the other hand, analysis of cell number distribution in blastocysts using the chi-square test indicated a significantly decreased cell proliferation in the experimental group.

Despite the harmful impact of deprenyl on rat preimplantation embryo development, deprenyl administration significantly decreased the DNA damage in blood lymphocytes as was scored employing Comet Assay. Our description of the adverse effects of deprenyl administration on rat preimplantation embryo development compared to the protective effects on the lymphocyte DNA is very important because deprenyl is still widely used in human medicine as a treatment. Potential mechanisms mediating deprenyl-induced impaired preimplantation embryo development are proposed.

Key words: MAO-B inhibitor — Preimplantation embryo — DNA damage

Introduction

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

In recent years, increasing numbers of claims have been made about the therapeutic effects of MAO inhibitors in psychiatric and neurological disorders, including depression, bulimia, schizophrenia, Parkinson's disease, neurodegenerative diseases in general, Alzheimer's disease etc. Although the molecular bases of these diseases are often complex, the fact that most of them have been linked with abnormal MAO activity provides a biochemical rationale for further pursuit of the therapeutic potential of MAO inhibitors. Synthesis of new MAO inhibitors could dramatically increase the effectiveness of the treatment and decrease the risk of undesirable side effects (Keung 2002).

If a woman becomes pregnant while being treated with an MAO inhibitor, she should be advised that there is little in-

Correspondence to: Jozef Mihalik, Department of Anatomy, Medical Faculty, P. J. Šafárik University, Šrobárova 2, 040 01 Košice, Slovakia
E-mail: jozef.mihalik@upjs.sk

formation about the safety of this drug during pregnancy and she should weight the benefit versus unknown risks carefully (Einarson 2005). The total lack of information as to whether long-term MAO inhibitor administration can influence the course of pregnancy during the preimplantation period of embryo development prompted us to start an investigation into this area. We have decided to begin our work employing the potent MAO-B inhibitor deprenyl which is known for its neuroprotective effects (Semkova et al. 1996), antiapoptotic effect in both cultured neurons (Le et al. 1997) and animal models (Simon et al. 2001) and exerting a cardiac neuroprotective effect in congestive heart failure (Hare 2001). On the other hand, Magyar and Szende (2004) found that deprenyl in high concentration (10^{-3} mol/l) induced apoptosis in tissue cultures of neuroectodermal origin (PC12, M1, M2058). Similarly, Szende et al. (2001) found that deprenyl has a biphasic effect. Serum deprivation caused apoptosis of the cultured A-2058 human melanoma cells, which could be decreased by administration of 10^{-9}–10^{-13} mol/l (–)-deprenyl. But high dose of deprenyl (10^{-3} mol/l) induced apoptosis and very high caspase 3 activity in the non-serum-deprived A-2058 culture cells. To extend the investigated area and to confirm our prediction that long-term administration of high deprenyl doses could increase DNA damage, an alkaline version of Comet Assay (revealing single-double-strand breaks of the DNA molecule as well as alkali-labile sites) was used to study DNA in blood lymphocytes.

Materials and Methods

Animals

All procedures performed with animals adhered to the permission of the Committee for Ethical Control of Animal Experiments at Šafárik University and the permission of the State Veterinary and Food Administration of the Slovak Republic (permission No. 7881/04-220/3). All efforts were made to minimize both the number of animals and their suffering.

Young, virgin female Wistar rats (200–240 g, 85–90 days old) were obtained from the animal facility of the Šafárik University. The animals were given free access to standard diet and water and were maintained in a 12 h light/12 h dark cycle. Rats were injected intraperitoneally daily for 30 days, either with saline (control animals) or with the dose (2.5 mg/kg/day) of deprenyl (M003, Sigma) dissolved in saline (experimental animals). After the last drug administration, females were mated during the following week.

Mating

Rodents, especially mice, are usually mated overnight and examined next morning to control the presence of a vaginal plug. But in rats, vaginal plug is very often already dissolved by the morning (own observations) and the time consuming procedure of finding sperm positivity in vaginal smears is needed. To avoid this, females were mated for 2 h only (vaginal plug is still easily visible) from 07:00–09:00 a.m. with males of the same strain. The first day on which a vaginal plug was present was designated day 1 of pregnancy.

Embryo collection

Pregnant rats were killed by lethal dose of thiopental (40 mg/kg; ICN Czech Pharma, Prague) 102–104 h after fertilization at 13:00 on day 5 of pregnancy and their blood was collected by cardiac puncture (see below). Oviducts and uterine horns were immediately removed and embryos were gently flushed from them with prewarmed PBS + PVP (3 mg/ml). Embryos were counted and classified according to developmental stage (Pampfer et al. 1990) as follows: a) degenerated – abnormal or damaged embryos with cytoplasmic fragmentation, b) morula, c) early blastocyst – with small blastocoele, d) expanded blastocyst – with a clearly demarcated trophoblast and inner cell mass, e) hatched blastocyst.

Cell staining

The total cell number of individual morulae and blastocysts was counted as was described before (Mihalik et al. 2000). Briefly, the *zona pellucida* was removed using short incubation in acid Tyrode solution (pH 2.3–2.4) and embryos were transferred onto a glass slide into small drops of working solution. The fluorescent DNA-specific dye 4'6-diamidino-2-phenylindole (DAPI, Sigma) was employed for nucleus staining by combining 0.75 ml of 80 mmol·l^{-1} sodium citrate dihydrate (Fisher Slovakia), 0.25 ml ethanol and 1 µl DAPI water stock solution (1.5 mg·ml^{-1}). The excess of working solution was removed and embryos were embedded between a slide and coverslip with Mowiol 4-88 (Calbiochem). Cell number counting was performed using UV light epifluorescence (Jenalumar a/d contrast, Carl Zeiss Jena, Germany).

Blood collection and lymphocytes isolation

1–2 ml of heparinised whole blood were collected by cardiac puncture from anaesthetised animals and delivered immediately to the laboratory. Most samples of blood were free from haemolysis and only such blood was used for lymphocytes isolation. Comet Assay was carried out within 1 h of blood collection. Lymphocytes were isolated from 150 µl of whole blood dissolved in phosphate buffered saline (PBS, pH 7.4, 4°C, dilution 1 : 4, Sigma) by density centrifugation (2500 rpm, 5 min, 4°C) on Ficoll-Paque^{TM} Plus gradient (Amersham Pharmacia Biotech AB, Sweden). Collected lymphocyte layers were rewashed in PBS (1 : 4) in the same conditions.

Single cell gel electrophoresis (Comet Assay)

All chemicals were purchased from Sigma. The Comet Assay was performed according to Singh et al. (1988) with minor modifications. Normal melting point (NMP) agarose and low melting point (LMP) agarose were dissolved in PBS (pH 7.4) using a microwave. Briefly, 50 µl of lymphocyte suspension (4×10^5 cells/ml) mixed with 1% LMP was added onto microscope slides precoated with 1% NMP agarose and allowed to solidify with cover slips in a refrigerator for 5 min. After solidification of the gel, the cover slips were removed and the slides were submersed in the lysing solution (2.5 mol/l NaCl, 100 mmol/l Na_2EDTA, 10 mmol/l Tris, 1% Triton X-100, pH 10) for 1 h. For assessing of single strand breaks of lymphocytes DNA (alkaline version of single cell gel electrophoresis), the slides were then placed in an unwinding buffer (300 mmol/l NaOH, 1 mmol/l Na_2EDTA, pH > 13) for 40 min and electrophoresis was carried out using the same solution for 20 min at 25 V and 300 mA. After electrophoresis, the slides were neutralised with a neutralization buffer (400 mmol/l Tris, pH 7.5, 15 min). All previously described steps were carried out in a refrigerator (4°C) (Singh et al. 1988).

Determination of lymphocyte DNA damage

The slides were stained with approximately 50 µl of SYBR Green solution and observed and scanned with an Olympus BX51 fluorescence microscope equipped with digital camera DP50. Lymphocytes DNA damage was examined by two parameters: % DNA in tail (= 100 – % DNA in head) and olive tail moment (= (tail mean – head mean) × % DNA in tail/100), using CometScore^{TM} 1.5 image analysis system (TriTek Corp., USA). For each individual sample, two slides were prepared and each 50 randomly chosen cells (total 100 cells) were selected.

Statistical analysis

Mean numbers of embryos, the cell number in morulae and blastocysts and % DNA in tail and olive tail moment in Comet Assay were analyzed by two-tailed unpaired Student's *t*-test. Results are given as means ± S.D. Differences in the distribution of preimplantation embryos and the cell number distribution in blastocysts were compared by the chi-square test. $p < 0.05$ was considered as significant. Data presented here are pooled from three independent replications of the same experiment.

Results

Embryo collection

The number of embryos isolated from animals is given in Table 1 and their developmental stage in Table 2. The mean number of embryos recovered per female differed significantly ($p < 0.05$) between the experimental and control groups (7.76 ± 3.01 vs. 9.63 ± 1.96, respectively). Moreover, almost 14% of embryos in the experimental group were flushed from oviducts compared with 1.95% of those from the control group ($p < 0.001$). Most of them were degenerated embryos, the lesser part consisted of morulae and early blastocysts. The morphological analysis of embryos isolated from deprenyl-treated females revealed significantly disturbed development ($p < 0.01$) because of the higher incidence of degenerated embryos and the lower incidence of morulae and blastocysts. But no significant changes ($p > 0.05$) were detected comparing developmental stages of blastocysts.

Cell number

No significant effect of deprenyl administration on the mean cell number in morulae or even blastocysts was found ($p > 0.05$; Table 3). On the other hand, statistical analysis employing chi-square test revealed decreased cell proliferation in

Table 1. Number of embryos collected from oviducts and uteri at day 5 of pregnancy from deprenyl-treated (2.5 mg/kg) and control rats

	Deprenyl	Control
No. of rats	17	16
total No. of embryos	132 (100%)	154 (100%)
embryos/rat ± S.D.	7.76 ± 3.01	9.63 ± 1.96
unpaired *t*-test	$p < 0.05$	
No. of embryos :		
flushed from oviducts	18 (13.64%)	3 (1.95%)
flushed from uteri	114 (86.36%)	151 (98.05%)
chi-square test	$p < 0.001$	

Table 2. Developmental stages of embryos and distribution pattern of blastocysts collected at day 5 of pregnancy from deprenyl-treated (2.5 mg/kg) and control rats

	Deprenyl	Control
No. of blastocysts	61 (46.21%)	76 (49.35%)
No. of morulae	39 (29.55%)	54 (35.07%)
No. of degenerated embryos	32 (24.24%)	24 (15.58%)
chi-square test	$p < 0.01$	
No. of hatched blastocysts	3 (4.92%)	6 (7.89%)
No. of expanded blastocysts	34 (55.74%)	39 (51.32%)
No. of early blastocysts	24 (39.34%)	31 (40.79%)
chi-square test	$p > 0.05$	

blastocysts derived from experimental females in comparison with the control group ($p < 0.001$; Table 3).

DNA damage

We have found that deprenyl administration significantly decreased the DNA damage in blood lymphocytes as was scored using Comet Assay (Table 4). Moreover, the decrement of DNA damage in experimental group was more than 44.1% comparing control group in the parameter % DNA in tail ($p < 0.001$) and more than 57.8% in the parameter olive tail moment ($p < 0.05$), respectively.

Discussion

Our results demonstrated that the long-term intraperitoneal administration of deprenyl to rat females significantly decreased the number of isolated embryos. Moreover, we have recorded significantly delayed embryo transport from oviduct to uterus and impaired embryonic development as was indicated by the increase in degenerated embryos and by the decrease in morulae and blastocysts. In contrast to this, deprenyl had no detrimental effect on the mean cell number in healthy morulae and blastocysts. However, analysis of cell proliferation revealed a higher percentage of blastocysts with a lower cell number in the experimental group. On the other hand, deprenyl administration had a significantly protective effect on lymphocyte DNA compared to its detrimental impact on preimplantation embryos.

The lower number of isolated embryos from experimental rats after MAO-B inhibitor (deprenyl) treatment could be explained in two ways. The first is mediated through impaired hypothalamic control of ovarian action and the second one by direct impact on ovarian ovulation. Substantial activity of MAO-B, using a coupled peroxidatic technique, was identified in several hypothalamic regions such as the periventricular and paraventricular nuclei (Willoughby et al. 1988). The hypothalamus regulates the sexual behavior of rats and works in the neural control of ovulation. The MAO value in the hypothalamus is the highest of all areas of the brain. This suggests the presence of high levels of biogenic amines in this area (Kono et al. 1994).

MAO activity, which is involved in the inactivation of catecholamines and serotonin *in vivo*, is detected in various reproductive organs. As was previously known, MAO-A type in the rat ovary is located predominantly in the corpora lutea and interstitial gland cells. But only MAO-B type was found in the ovarian blood vessels, where it has shown characteristic changes of activity during the oestrous cycle and was higher in the proestrus. These histochemical results suggest that MAO-B activity might possibly be involved in ovulation, presumably in association with humoral information (Yoshimoto et al. 1986).

More than 98% of control embryos were collected from the uterus compared to 86% embryos from experimental animals. The oviduct can distinguish between unfertilised oocytes and preimplantation embryos which are transported at different rates, with embryos reaching the uterus one day earlier than unfertilised oocytes (Talbot and Riveles 2005). More degenerated embryos were collected from experimental animals than from controls. But the ratio between them (1.33 : 1) does not correlate with the ratio between experimental and control embryos flushed from oviducts (6 : 1). It is clear that some other(s) factor(s) must be involved in the delayed embryo transport in our rat females. Recently, the discovery of both MAO enzymes in the oviduct's *lamina propria mucosae* layer and predominantly in the muscle cells of rat oviduct and uterus have been documented (Mihalik et al. 2004). Thus, one can speculate that administration of MAO-B inhibitor can disturb the passage of the embryo through the female reproductive system.

L-deprenyl, a potent MAO-B inhibitor, has been used clinically as an antidepressant for many years (Singer and

Table 3. Cell number in morulae and blastocysts and distribution of cell number in blastocysts (DAPI staining) isolated from deprenyl-treated (2.5 mg/kg) and control rats

	Deprenyl	Control
No. of stained morulae	21	30
cell No. ± S.D.	14.00 ± 2.51	15.10 ± 3.03
unpaired *t*-test	$p > 0.05$	
No. of stained blastocysts	41	66
cell No. ± S.D.	23.68 ± 8.17	23.59 ± 5.17
unpaired *t*-test	$p > 0.05$	
No. of stained blastocysts	41	66
16–24 cells	63.41%	53.03%
25–32 cells	29.27%	39.39%
>32 cells	7.32%	7.58%
chi-square test	$p < 0.001$	

Table 4. DNA damage in blood lymphocytes using Comet Assay from deprenyl-treated (2.5 mg/kg) and control rats

	Deprenyl	Control
No. of rats	12	9
% DNA in tail	14.75 ± 3.91	26.40 ± 6.93
unpaired *t*-test	$p < 0.001$	
olive tail moment	2.12 ± 0.87	5.03 ± 3.75
unpaired *t*-test	$p < 0.05$	

Ramsay 1995). Cigarette smoking has a similar effect, due to the presence of an MAO inhibitory compound in tobacco smoke. The observation that smokers have reduced brain MAO-A and MAO-B levels impels the need to look beyond nicotine as the only pharmacologically relevant substance in tobacco smoke (Fowler et al. 2003). Concerning reproduction we have to keep in mind that the transport of preimplantation embryos through the oviduct can be inhibited by cigarette smoking, apparently by an inhibition of oviductal smooth muscle contraction (Talbot and Riveles 2005). Certain tobacco constituents, but not nicotine, have modest MAO-inhibitory activity. The β-carboline compound norharman is a prominent candidate as an inhibitor of MAO-B in tobacco smoking. In contrast to norharman, the β-carboline harman is not a potent inhibitor of MAO-B, but effectively inhibits MAO-A. Finally, nitric mono-oxide (NO), a major component of cigarette smoke, has an MAO-inhibiting activity but is not involved in the inhibition of MAO in tobacco smokers since this radical is rapidly bound in the circulation by haemoglobin and degraded to nitrite (van Amsterdam et al. 2006). From this point of view there is a need to look at cigarette smoking and deprenyl administration as having a possible detrimental effect on preimplantation embryo development through impaired MAO enzyme levels. Nevertheless, futher experiments are necessary to prove negative impact of MAO inhibitory compounds in tobacco smoke on the process of reproduction.

Interestingly, we were not able to prove the detrimental effect of deprenyl administration on the mean cell number in morulae or even blastocysts. The mean cell number in blastocysts was in very close agreement with the values described by Spielmann et al. (1980) but was slightly lower than the values found by Pampfer et al. (1990). This could be explained by use of a very similar mating scheme (Spielmann et al. 1980) as was employed in our experiment, when the precise time of copulation, fertilization and subsequently the time of embryo development was determined for each female. In contrast to results achieved by Student's *t*-test, analysis employing the more sensitive chi-square test confirmed significantly decreased cell proliferation in blastocysts derived from experimental animals. It may be a good point of interest to observe latterly whether those delayed cell divisions have some consequent effect on the number of implanting embryos or/and even on birth weight. But other types of experiments are needed to confirm this hypothesis.

Since Iľková et al. (2004) have proven the expression of serotonin 5-HT1D receptor mRNA from mouse oocytes and preimplantation embryos to the blastocyst stage it appears that endogenous and/or exogenous serotonin in preimplantation embryos could be involved in the regulation of embryo development and embryo-maternal interactions. Deprenyl is well known for its capability to inhibit not only MAO-B but also MAO-A in specific regions of the rat brain, an enzyme which preferentially oxidises serotonin (Lakshmana et al. 1996). To date there has been no relevant published work describing the influence of deprenyl administration on serotonin and MAO-A levels in the reproductive system. Due to this, we should not eliminate the possibility that the impaired embryo development described in our paper was achieved through the disturbed MAO-A level in the oviduct/uterus, because mice embryo exposure to serotonin *in vitro* had a detrimental effect (Iľková et al. 2004).

Over the past decade, the Comet Assay has become one of the standard methods for assessing DNA damage, with applications in genotoxicity testing, human biomonitoring and molecular epidemiology, ecogenotoxicology, as well as fundamental research in DNA damage and repair (Collins 2004). From this point of view it is interesting that even the high dose of deprenyl employed in our experiment can stimulate the decrease in DNA damage in blood lymphocytes. Similarly, Maruyama and Naoi (1999) state strong anti-apoptotic effect of deprenyl on dopaminergic SH-SY5Y cells determined by a Comet Assay. Its protective action lasted even after it was washed out, suggesting that it may initiate the intracellular process to repress the apoptotic death program.

Lymphoid organs are innervated extensively by noradrenergic sympathetic nerve fibers. Lymphocytes, macrophages, and other cells of the immune system bear functional adrenoreceptors. Pharmacological manipulation of noradrenergic innervation in lymphoid organs has confirmed that norepinephrine can modulate cell-mediated and humoral immunity. These findings illustrate the importance of the sympathetic nervous system in modulating immune function under normal and disease states (Madden et al. 1995). ThyagaRajan et al. (1999) determined that deprenyl administration elevated concavalin A-induced T lymphocyte proliferation following 30 days of treatment in rats. Moreover, neurorestorative property on sympathetic noradrenergic innervation of the spleen was recorded, which may leads to an improvement of immune responses. Considering this we can not exclude, that protective effect of deprenyl administration on lymphocyte DNA detected in our work could be mediate at least partially indirectly by modulation of sympathetic innervation and norpinephrine levels in lymphoid organs.

Despite the fact that deprenyl administration decreased DNA damage in blood lymphocytes at dose 2.5 mg/kg over 30 days in rats, its negative impact on the process of reproduction has been recorded. The number of collected embryos was lower but the incidence of degenerated embryos was higher in the experimental group. Moreover, we have found delayed cell proliferation in blastocysts. Due to this, it could be dangerous in humans, and especially in young women who want to achieve pregnancy, to use deprenyl as a treatment at high doses over a long period. The data presented here will be used as the basis for further experiments

in the research into the influence of MAO inhibitors on the reproductive system in rats and humans. To our knowledge, this is the first paper describing the detrimental effect of MAO inhibitor administration on the preimplantation embryo development in mammals.

Acknowledgements. This research work was supported in part by the VEGA grant 1/4227/07 and in part by the VEGA grant 1/4233/07.

References

Collins A. R. (2004): The comet assay for DNA damage and repair: principles, applications, and limitations. Mol. Biotechnol. **26**, 249–261

Einarson A. (2005): The safety of psychotropic drug use during pregnancy: A review. From Medscape General Medicine

Fowler J. S., Logan J., Wang G.-J., Volkow N. D. (2003): Monoamine oxidase and cigarette smoking. Neurotoxicology **24**, 75–82

Hare J. M. (2001): Oxidative stress and apoptosis in heart failure progression. Circ. Res. **89**, 198–200

Iľková G., Rehák P., Veselá J., Čikoš Š., Fabian D., Czikková S., Köppel J. (2004): Serotonin localization and its functional significance during mouse preimplantation embryo development. Zygote **12**, 205–213

Keung W. M. (2002): Monoamine oxidase inhibitors. Expert Opin. Ther. Pat. **12**, 1813–1829

Kono H., Lin Y. C., Yamaguchi M., Zuspan F. P., O'Shaughnessy R. W., Lee A. C., Furuhashi N., Yokaichiya T., Takayama K., Yajima A. (1994): Monoamine oxidase activity in rat organs during pregnancy. Tohoku J. Exp. Med. **172**, 1–8

Lakshmana M. K., Jagadeesh S., Subhash M. N. (1996): Increased acetylcholinesterase activity in selected regions of rat brain after chronic (–)-deprenyl administration. Neurochem. Res. **21**, 1209–1212

Le W., Jankovic J., Xie W., Kong R., Appel S. (1997): (–)-deprenyl protection of 1-methyl-4 phenylpyridium ion (MPP^+)-induced apoptosis independent of MAO-B inhibition. Neurosci. Lett. **224**, 197–200

Madden K. S., Sanders V. M., Felten D. L. (1995): Catecholamine influences and sympathetic neural modulation of immune responsiveness. Ann. Rev. Pharmacol. Toxicol. **35**, 417–448

Magyar K., Szende B. (2004): (–)-deprenyl, a selective MAO-B inhibitor, with apoptotic and anti-apoptotic properties. Neurotoxicology **25**, 233–242

Maruyama W., Naoi M. (1999): Neuroprotection by (–)-deprenyl and related compounds. Mech. Ageing Dev. **111**, 189–200

Mihalik J., Rehak P., Koppel J. (2000): The influence of insulin on the *in vitro* development of mouse and bovine embryos. Physiol. Res. **49**, 347–354

Mihalik J., Schmidtová K., Dorko F., Kočišová M. (2004): Histochemical detection of MAO in rat female reproductive organs. In: Proceedings of 42nd Congress of the Slovak Anatomical Society with International Participation (June 28 – July 1), Košice, Slovakia

Pampfer S., Vanderheyden I., Michiels B., De Hertogh R. (1990): Cell allocation to the inner cell mass and the trophectoderm in rat embryos during *in vivo* preimplantation development. Roux's Arch. Dev. Biol. **198**, 257–263

Semkova I., Wolf P., Schilling M., Krieglstein J. (1996): Selegiline enhances NGF synthesis and protects central nervous system neurons from excitotoxic and ischemic damage. Eur. J. Pharmacol. **315**, 19–30

Simon L., Szilagyi G., Bori Z., Orbay P., Nagy Z. (2001): (–)-deprenyl attenuates apoptosis in experimental brain ischemia. Eur. J. Pharmacol. **430**, 235–241

Singer T. P., Ramsay R. R. (1995): Monoamine oxidases; old friends hold many surprises. FASEB J. **9**, 605–610

Singh N. P., McCoy M. T., Tice R. R., Schneider E. L. (1988): A simple technique for quantitation of low levels of DNA damage in individual cells. Exp. Cell Res. **175**, 184–191

Spielmann H., Jacob-Müller U., Beckord W. (1980): Immunosurgical studies on inner cell mass development in rat and mouse blastocysts before and during implantation *in vitro*. J. Embryol. Exp. Morphol. **60**, 255–269

Szende B., Bökönyi G., Bocsi J., Kéri G., Timár F., Magyar K. (2001): Anti-apoptotic and apoptotic action of (–)-deprenyl and its metabolites. J. Neural. Transm. **108**, 25–33

Talbot P., Riveles K. (2005): Smoking and reproduction: the oviduct as a target of cigarette smoke. Reprod. Biol. Endocrinol. **3**, 52

ThyagaRajan S., Madden K. S., Stevens S. Y., Felten D. L. (1999): Effects of L-deprenyl treatment on noradrenergic innervation and immune reactivity in lymphoid organs of young F344 rats. J. Neuroimmunol. **96**, 57–65

van Amsterdam J., Talhout R., Vleeming W., Opperhuizen A. (2006): Contribution of monoamine oxidase (MAO) inhibition to tobacco and alcohol addiction. Life Sci. **79**, 1969–1973

Willoughby J., Glover V., Sandler M. (1988): Histochemical localisation of monoamine oxidase A and B in rat brains. J. Neural Transm. **74**, 29–42

Yoshimoto Y., Sakumoto T., Arai R., Miyake A., Kimura H., Aono T., Tanizawa O., Maeda T. (1986): Monoamine oxidase in rat ovary during the estrous cycle. A histochemical study by a new coupled peroxidatic oxidation method. Endocrinology **119**, 1800–1804

Final version accepted: May 15, 2008