Effect of Deprenyl on Free Radical Oxidation in Rat Brain during Immobilization Stress

V. E. Tseilikman, A. I. Sinitsky, K. A. Poyarkov, and E. V. Vojdaev

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 148, No. 12, pp. 615-617, December, 2009
Original article submitted March 5, 2009

Rarely repeated episodes of 1-h immobilization in rats were accompanied by an increase in the content of molecular products of lipid peroxidation and decrease in the amount of oxidatively modified proteins. Monoamine oxidase B inhibitor deprenyl prevented the poststress activation of lipid peroxidation.

Key Words: *stress; monoamine oxidase B; lipid peroxidation; protein oxidation; deprenyl*

Free radical oxidation is involved in the pathogenesis of vascular and neurodegenerative diseases and plays a role in the development of age-related injury to the central nervous system (CNS) [3,5,7]. Previous studies showed that monoamine oxidase B (MAO-B) inhibitor deprenyl reduces the severity of oxidative stress and produces a neuroprotective effect during Alzheimer's disease [12]. A relationship exists between the content of molecular products of lipid peroxidation (LPO) and activity of MAO-B in human CNS during postnatal ontogeny [5,6]. Published data show that similar variations are observed during rarely repeated episodes of 1-h immobilization (RRIM) [10]. These data suggest that activation of LPO in the brain is a MAO-B-dependent process. Here we tested this hypothesis.

MATERIALS AND METHODS

Experiments were performed on male and female outbred rats (n=31). The animals were divided into groups. Group 1 consisted of control animals. Group 2 animals were exposed to RRIM (1-h fixation of animals in the supine position on a plywood plate). The intervals between the stress episodes were 72 h. The procedure was repeated 4 times. This model of chronic stress is accompanied by a decrease in the resistance to acute hypoxic hypoxia, development of anxiety-depression disorders, activation of cerebral MAO-B, and accumulation of LPO products in CNS.

Group 3 rats received selective MAO-B inhibitor deprenyl (Jumex, Sanofi/Chinoin) in a dose 10 mg/kg. Deprenyl was administered 1 h before each episode of restraint stress. A special series was conducted to evaluate the dose of deprenyl providing stable inhibition of cerebral MAO-B in adult outbred rats. The initial doses of deprenyl were selected from the single therapeutic dose of this drug in humans taking into account the differences in the relative body surface area [1]. The minimum dose of deprenyl was 50% of the estimated equivalent of an average therapeutic dose (EATD). The maximum dose of this drug exceeded EATD by 16 times. MAO-B activity was measured after treatment with deprenyl in doses of 0.625, 1.25, 2.5, 5, 10, and 20 mg/kg. Deprenyl in a dose of 10 mg/kg was most effective, which agrees with published data [11]. The regimen of drug treatment was selected to cause persistent inhibition of MAO-B for 14 days.

Group 4 consisted of unstressed animals receiving the same dose of deprenyl. The treatment regimen in group 4 rats was similar to that in animals of the RRIM group.

The rats were euthanized under ether anesthesia 72 h after the last stress exposure. This period corresponds to simultaneous activation of MAO-B and LPO in the brain [10]. Activity of MAO-B [4] and contents of primary (conjugated dienes) and secondary molecu-

Chelyabinsk State Medical Academy, Russian Ministry of Health, Russia. ***Address for correspondence:*** vadimed@yandex.ru. V. E. Tseiliknam

0007-4888/09/1486-0856

lar products of LPO (ketodienes and conjugated trines) were measured in brain homogenates [2]. The amount of end products of LPO (Schiff bases) was estimated [9]. The content of oxidatively modified (carbonylated) proteins was measured as described previously [8]. The measurements were performed simultaneously. The results were analyzed by methods of variation statistics. The data are expressed as the arithmetic mean and standard error of the mean ($M \pm m$). The differences were evaluated by nonparametric tests (Mann—Whitney *U* test; Wald—Wolfowitz *WW* test; and one-sided Kolmogorov—Smirnov test, λ). The results were analyzed by Statistica 6.0 software.

RESULTS

Activity of cerebral MAO-B and intensity of LPO were shown to increase 72 h after RRIM. This manifested in an increase in the content of isopropanol-soluble and heptane-soluble ketodienes, conjugated dienes, and heptane-soluble Schiff bases in the brain (Table 1). Oxidative destruction of proteins was reduced under these conditions: the content of oxidatively modified proteins decreased from 0.32±0.02 (control, n=8) to 0.18±0.02 mmol/g protein (72 h after RRIM, n=8; p=0.006, *U* test). Hence, repeated stress produced opposite effects on these components of free radical oxidation.

Administration of deprenyl to unstressed animals was followed by inhibition of cerebral MAO-B, increase in the contents of isopropanol-soluble ketodienes and conjugated trienes, and decrease in the content of heptane-soluble Schiff bases (Table 1). Therefore, the decrease in activity of MAO-B in unstressed animals is accompanied by changes in the kinetics of LPO (inhibition of final stages of LPO). The inhibition of cerebral MAO-B was followed by a decrease in the content of carbonylated proteins upon induction in the Fe^{2+}–H_2O_2 system. The content of oxidatively modified proteins in the brain of control animals (n=8) and deprenyl-receiving rats (n=8) was 3.08±0.09 and 2.79±0.17 mmol/g protein respectively (p=0.038, *WW* test).

Administration of deprenyl to unstressed animals was accompanied by a decrease in activity of cerebral MAO-B and prevented poststress LPO activation. The content of isopropanol-soluble and heptane-soluble Schiff bases and heptane-soluble conjugated dienes in group 3 rats was much lower than in group 2 animals (Table 1). MAO-B activation during RRIM was accom-

TABLE 1. Effect of RRIM and Deprenyl on MAO-B Activity and Content of Molecular Products of LPO in Rat Brain

Parameter	Group			
	1, control (n=8)	2, RRIM (n=8)	3, RRIM+deprenyl (n=8)	4, deprenyl (n=8)
MAO-B activity, nmol/g protein/min	0.11±0.01	0.130±0.003 $P_{1,2}$=0.047*U*	0.077±0.005 $P_{2,3}$=0.003*U*	0.089±0.006 $P_{1,4}$=0.003*U*
Conjugated dienes (heptane phase)	0.452±0.024	0.500±0.016	0.438±0.038 $P_{2,3}$=0.038*WW*	0.410±0.031
Ketodienes and conjugated trienes (heptane phase)	0.087±0.004	0.110±0.008	0.090±0.011 $P_{1,2}$=0.012*U*	0.099±0.003 $P_{4,3}$=0.038*WW*
Schiff bases (heptane phase)	0.049±0.005	0.059±0.010	0.034±0.005 $P_{2,3}$=0.036*U*	0.034±0.006 $P_{1,4}$=0.036*U*
Ketodienes and conjugated trienes (isopropanol phase)	0.072±0.015	0.132±0.024 $P_{1,2}$=0.027*U*	0.125±0.017	0.123±0.015 $P_{1,4}$=0.046*U*
Schiff bases (isopropanol phase)	0.020±0.007	0.034±0.006	0.017±0.004 $P_{2,3}$=0.027*U*	0.033±0.011
Conjugated dienes (isopropanol phase), induction with Fe^{2+}-ascorbate	1.052±0.013	1.035±0.030 $P_{1,2}$=0.002*WW*	1.022±0.024 $P_{4,3}$=0.038*WW*	1.010±0.007 $P_{1,4}$=0.012*U*

Note. The content of LPO products is expresses in arbitrary units of the oxidation index. $P_{1,2}$, statistically significant differences between groups 1 and 2; $P_{1,4}$, statistically significant differences between groups 1 and 4; $P_{2,3}$, statistically significant differences between groups 2 and 3; $P_{4,3}$, statistically significant differences between groups 4 and 3.

panied by different changes in the intensity of LPO and oxidative modification of proteins. Similar variations were revealed in various regions of human brain during postnatal development [3]. The decrease in oxidative destruction of proteins and against the background of LPO activation is probably associated with effective antioxidant protection of proteins, which manifested in increased oxidizability of brain proteins determined as the ratio of the content of carbonylated proteins after induction to their basal content (control–RRIM).

Thus selective MAO-B inhibitor deprenyl significantly reduces post-stress accumulation of molecular products of LPO, which confirms our hypothesis. It should be noted that we observed a decrease in the intensity of not only basal LPO, but also induced LPO charactering the efficiency of antioxidant protection (number of unsaturated acyl bonds that serve as the substrate for peroxidation). Similar results were obtained in groups 2 and 4 (Table 1). In this context it should be noted that RRIM is characterized by desensitization to glucocorticoids. Corticosterone is known to induce desaturation of fatty acids, thus replenishing the pool of LPO substrates. It can be hypothesized that the decrease in the intensity of induced LPO produced by RRIM is determined by inhibition of glucocorticoid-dependent desaturation of fatty acids. The same effect in group 4 animals can be related to metabolic transformation of this compound in the liver by the CYP3A isoform of cytochrome P450. This isoform is also involved in terminal biotransformation of glucocorticoids. Deprenyl in high dose probably potentiates overproduction of CYP3A, which abolishes the effect of glucocorticoids.

This was supported by the Russian Foundation for Basic Research (grant No. 08-04-01526-a).

REFERENCES

1. I. A. Volchegorskii, I. I. Dolgushin, O. L. Kolesnikov, and V. E. Tseilikman, *Experimental Modeling and Laboratory Study of Adaptive Reactions in the Organism* [in Russian], Chelyabinsk (2000).
2. I. A. Volchegorskii, R. I. Lifshits, A. G. Nalimov, and B. G. Yarovinskii, *Vopr. Med. Khimii*, No. 1, 127-131 (1989).
3. I. A. Volchegorskii, N. V. Malinovskaya, O. V. Shumeleva, and S. E. Shemyakov, *Byull. Eksp. Biol. Med*., **144**, No. 8, 159-169 (2007).
4. I. A. Volchegorskii, N. A. Skoboleva, and R. I. Lifshits, *Vopr. Med. Khimii*, No 1, 86-89 (1991).
5. I. A. Volchegorskii, S. E. Shemyakov, I. B. Telesheva, *et al*., *Fiziol. Chel*., **31**, No. 2, 108-105 (2005).
6. I. A. Volchegorskii, S. E. Shemyakov, V. V. Turygin, and N. V. Malinovskaya, *Byull. Eksp. Biol. Med*., **132**, No. 8, 174 (2001).
7. E. I. Gusev and V. I. Skvortsov, *Cerebral Ischemia* [in Russian], Moscow (2001).
8. E. E. Dubinina, S. O. Burmistrov, D. A. Khodov, and I. G. Porotov, *Vopr. Med. Khimii*, **41**, No. 1, 24-26 (1995).
9. E. I. L'vovskaya, I. A. Volchegorskii, S. E. Shemyakov, and R. I. Lifshits, *Ibid*., **31**, No. 4, 92-94 (1991).
10. V. E. Tseilikman, I. A. Volchegorskii, O. B. Tseilikman, *et al*., *Ros. Fiziol. Zh*., **91**, No. 4, 394-398 (2005).
11. S. Knollema, W. Aukema, H. Hom, *et al*., *Stroke*, **26**, No. 10, 1883-1887 (1995).
12. M. Sano, C. Ernesto, R. G. Thomas, *et al*., *N. Engl. J. Med*., **336**, No. 17, 1216-1222 (1997).