# Effect of Pro- and Antioxidants on Insulin Sensitivity and Glucose Tolerance

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We studied the correlation between the effect of α-lipoic acid, emoxipin, reamberin, and mexidol on LPO *in vitro* and the action of these drugs on insulin sensitivity and tolerance to glucose load *in vivo*. It was found that the preparations producing prooxidant effect *in vitro* (α-lipoic acid and reamberin) are characterized by pronounced insulin-potentiating activity, but only slightly increase (α-lipoic acid) or even decrease (reamberin) tolerance to glucose load. 3-Hydroxypyridine derivatives (emoxipin and mexidol) producing an antioxidant effect *in vitro* increase glucose tolerance, but exhibit relatively weak insulin-potentiating activity. These results suggest that differential use of the studied drugs in patients with diabetes mellitus depending on the type of the disease and individual insulin requirement is a promising trend in medical studies.

**Key Words:** *insulin sensitivity; glucose tolerance; lipid peroxidation; α-lipoic acid; 3-hydroxypyridine and succinic acid derivatives*

The efficiency of insulin interaction with the corresponding receptor largely depends on intracellular mechanisms of redox-transduction of the hormonal signal. These mechanisms are aimed at amplification of the receptor-mediated tyrosine kinase cascade via reversible oxidative inhibition of protein tyrosine phosphatases [10]. Oxidation of sulfhydryl groups of insulin receptor β-chain is considered to be a mechanism of its redox priming promoting the increase in receptor tyrosine kinase activity upon its interaction with insulin [10]. Insulin-stimulated homologue of NADPH-oxidase (Nox4) and $Mn^{2+}$-dependent form of this enzyme are the sources of oxidants for this redox-dependent amplification of the hormonal signal [10,13]. Under conditions of diabetes mellitus, hyperglycemia promotes enhanced production of antioxidants and considerably potentiates NADPH-oxidase transduction of the insulin signal [10]. At the same time, long-term and excessive stimulation of the production of reactive oxygen species (ROS) can lead to the development of oxidative stress (OS) and the formation of insulin resistance [10]. Ambiguous role of ROS in the regulation of insulin sensitivity seriously complicates the problem of metabolic safety of OS correctors (including 3-hydroxypyridine and succinic acid derivatives emoxipin, reamberin, and mexidol manufactured in Russia and α-lipoic acid used for the treatment of diabetic neuropathies [1,4]) in the treatment of late complications of diabetes mellitus.

Here we studied the influence of these preparations on *in vivo* insulin sensitivity depending on their effect on LPO *in vitro*.

## MATERIALS AND METHODS

The following drugs were used: 1% emoxipin solution (2-ethyl-6-methyl-3-hydroxypyridine hydrochloride; Moscow endocrine plant), 1.5% reamberin solution (N-(1-deoxy-D-glucitol-1-yl)-N-methylammonium sodium succinate; Polisan), 5% mexidol solution (2-eth-

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yl-6-methyl-3-hydroxypyridine succinate, Ellara), and 2.5% α-lipoic acid solution (berlition, Berlin-Chemie).

The effect of the test drugs on LPO *in vitro* was evaluated by accumulation of TBA-reactive substances in 2% homogenates of rat brain incubated for 60 min at 37°C on open air [2]. The test drugs were added to standardized samples of nervous tissue homogenate immediately before incubation. LPO-modulating effects of each drug were studied in a concentration range from nano- to millimoles. The intensity of LPO in cerebral homogenates was evaluated by accumulation of TBA-reactive substances (% from the initial level). Each sample was processed in 5 parallel aliquots and the mean value was then calculated.

In experiments on evaluation of the drug effect on insulin sensitivity *in vivo* we used 140 rats deprived of food for 24 h before the experiment, but allowed free access to water. The test drugs were injected singly intraperitoneally. Each preparation was administered in 3 doses extrapolated from single therapeutic doses for humans with correction for different body surface areas [2]. In all cases, the minimum dose was equal to $^1/_2$ of the calculated equivalent of the mean therapeutic dose (EMTD). Doubled EMTD was used as the maximum dose. α-Lipoic acid was administered in doses of 25, 50, and 100 mg/kg, emoxipin in doses 6.25, 12.5, and 25 mg/kg, mexidol in doses 12.5, 25, and 50 mg/kg, and reamberin in doses 12.5, 25, and 50 ml/kg. Controls received equivalent volumes of 0.9% NaCl. Immediately after intraperitoneal injection of the test drugs or 0.9% NaCl, neutral insulin solution (Humulin Regular, Eli Lilly) was intravenously infused in a dose of 40 U/kg. Insulin sensitivity of experimental animals was evaluated by the latency of insulin coma [2].

In an additional experimental series, the effects of emoxipin, reamberin, mexidol, and α-lipoic acid on glucose tolerance were evaluated on 140 rats deprived of food for 24 h before the experiment, but allowed free access to water. Thirty minutes after single administration of the test drug in the specified doses, the blood was sampled from the caudal vein for recording the initial glycemia values and glucose tolerance test (GTT) was carried out. To this end, glucose load in a dose of 2 g/kg (5 ml/kg 40% glucose intraperitoneally) was performed. Glycemia level in the dynamics of GTT was measured after 60 and 120 min. Controls received an equivalent volume of 0.9% NaCl instead of the test drugs 30 min before GTT.

Statistical processing of the results was performed using SPSS-13.0 software. The data of *in vivo* experiments were processed by methods of descriptive statistics and presented as arithmetic mean±standard error. Significance of differences between unrelated samples was analyzed using Mann–Whitney test. The significance of differences between related samples was evaluated using paired Wilcoxon test. Interrelations between the parameters were evaluated by calculating Kendall correlation coefficients ($r_k$). Verification of statistical hypotheses was performed at $p$=0.05.

## RESULTS

The test drugs considerably differed by the effect on LPO intensity *in vitro* (Fig. 1). Reamberin and α-lipoic acid produced a pronounced prooxidant effect and markedly stimulated generation of TBA-reactive products in standardized homogenates of rat brain. In both cases, an appreciable prooxidant effect of these preparations was observed starting from micromolar concentrations and peaked at their millimolar concentrations. On the contrary, emoxipin and mexidol produced a pronounced antioxidant effect in the whole concentration range. It should be noted that mexidol, a derivative of both 3-hydroxypyridine and succinic acid, was inferior to emoxipin by LPO-limiting activity in concentrations of $10^{-9}$-$10^{-4}$ M and in millimolar concentrations even stimulated the formation of TBA-reactive products (Fig. 1). These findings agree with enhanced formation of ROS during mitochondrial oxidation of succinic acid in the nervous tissue [6,14]. It can be assumed that fragments of inner mitochondrial membranes present in non-fractionated homogenates of rat brain can provide intensive ROS generation upon addition of reamberin to the incubation medium. The differences in antioxidant activity of emoxipin and mexidol containing completely identical 3-hydroxypyridine component (2-ethyl-6-methyl-3-hydroxypyridine) can be determined by the same mechanism. Oxidation of the succinate component of mexidol probably reduces the antioxidant effect of 2-ethyl-6-methyl-3-hydroxypyridine. This effect was not reproduced in model systems excluding enzymatic oxidation of succinic acid [5]. It should be noted that mexidol in these systems was superior to emoxipin by its antioxidant activity.

These results confirm the opinion that regulators of free radical processes are often unreasonably assigned to antioxidants [6]. In our study this concerns not only derivatives of succininc acid, but also α-lipoic acid containing it in an oxidized form and exhibiting pronounced prooxidant activity due to the presence of a dithiolan ring [8]. Our experimental findings (Fig. 1) and published data [6] suggest that succinic and α-lipoic acids play a role of co-antioxidants, rather than true antioxidants in the antioxidant defense system. Of the studied drugs, only 3-hydroxypyridine derivatives (emoxipin and mexidol) can be considered as true antioxidants.

All studied preparations potentiated the effects of insulin (Table 1), but this activity considerably depended on the direction of their LPO-modulating ef-

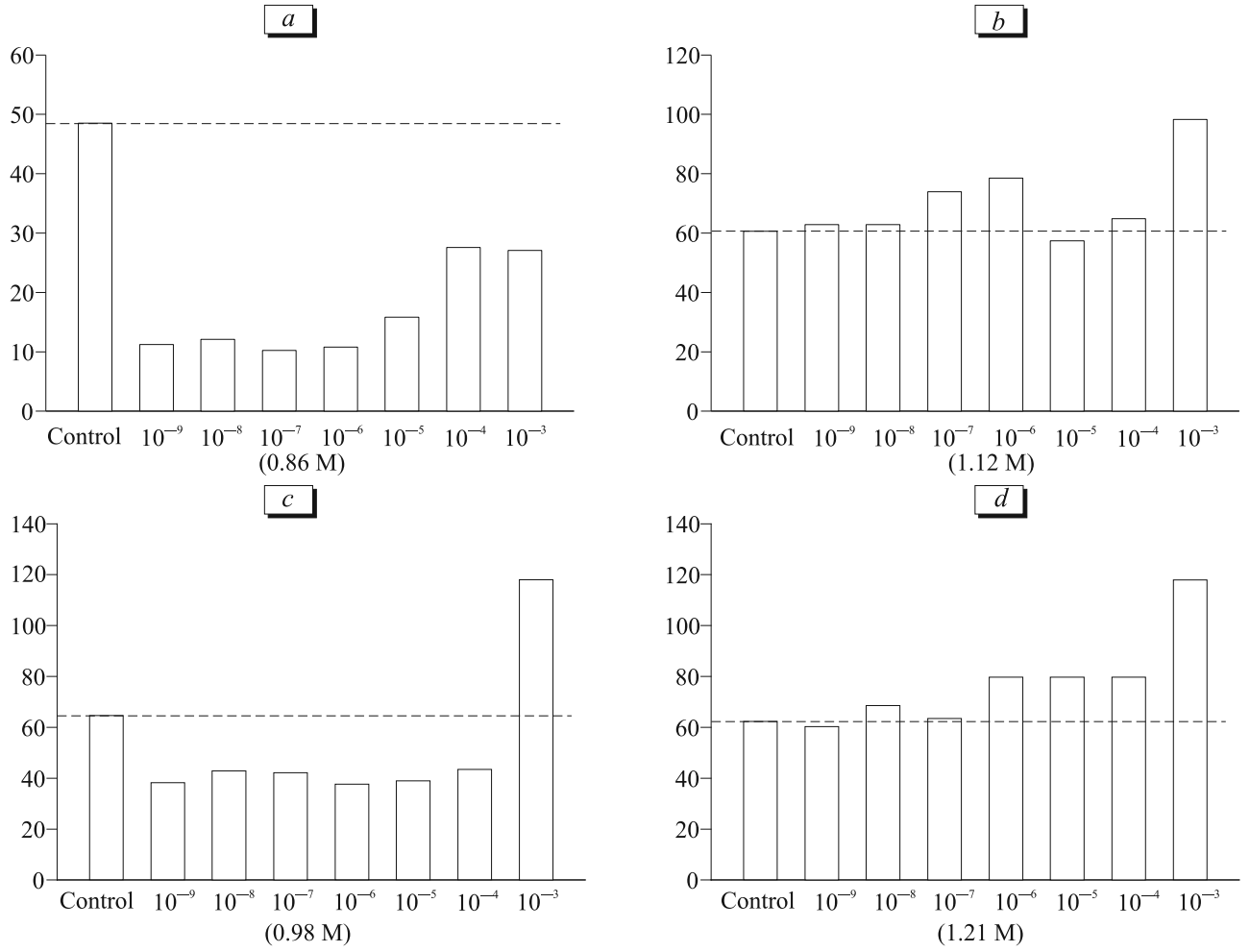


**Fig. 1.** Effect of emoxipin (*a*), reamberin (*b*), mexidol (*c*), and α-lipoic acid (*d*) on LPO in rat brain homogenate *in vitro*. Initial concentrations of the test substances in preparations are shown in parentheses. The incubation mixture contained the test drugs dissolved in 1.8 ml 0.04 M phosphate buffered saline prepared on 0.9% NaCl pH 7.4 and 0.2 ml 20% rat brain homogenate; incubation was performed for 60 min at 37°C. The data are presented as the mean of 5 repetitions *in vitro*. Ordinata: Accumulation of TBA-reactive substances, % of initial level.

fect. The drugs promoting accumulation of TBA-reactive substances in homogenates of rat brain *in vitro* most markedly reduced the latency of insulin coma *in vivo*. This was most clearly seen in case of α-lipoic acid: it 1.9-2.7-fold reduced the latency of insulin coma compared to the control values and this effect was dose-dependent. Similar results were obtained for reamberin: it dose-dependently accelerated the onset of insulin coma by 1.6-1.8 times. These findings agree with the data on direct correlation between functional activity of mitochondrial electron-transporting chains and insulin sensitivity [15]. In case of succinic acid derivative reamberin, this can be related to enhanced transport of reducing equivalents to the electron transport chain via SDH and concurrent intensification of $H_2O_2$ production by mitochondria. Succinate-induced accumulation of $H_2O_2$ promotes redox-priming of insulin receptor β-subunit thus potentiating the effect of insulin [10]. At the same time, long-term and excessive stimulation of $H_2O_2$ production can lead to the development of oxidative stress and the formation of insulin resistance [9]. This explains less pronounced insulin-potentiating effect of reamberin compared to that of α-lipoic acid, which acts as a prooxidant in the oxidized form and as an antioxidant in the reduced state [12]. Due to this peculiarity, α-lipoic acid can produce an insulin-potentiating effect (in oxidized state) and prevent the development of oxidative stress-dependent insulin-resistance (in reduced state).

Emoxipin and mexidol exhibited less pronounced insulin-potentiating activity non-linearly depending on the dose (Table 1). In both cases, the maximum and equally pronounced shortening of the latency of insulin coma (by 1.2 times compared to the control) was observed in the middle of the studied dose range (EMTD). EMTD of mexidol was the only dose producing significant insulin-potentiating effect. In case of emoxipin, the least pronounced (but significant)

**TABLE 1.** Effect of α-Lipoic Acid and 3-Hydroxypyridine and Succinic Acid Derivatives on Latency of Insulin Coma in Rats (*M*±*m*)

| Group | Latency of insulin coma, min |
|---|---|
| Emoxipin | |
| Control | 247.10±5.47 |
| $^1/_2$EMTD (6.25 mg/kg) | 226.90±8.36 |
| EMTD (12.5 mg/kg) | 209.70±8.08* |
| 2EMTD (25 mg/kg) | 220.20±12.35* |
| Reamberin | |
| Control | 236.50±5.49 |
| $^1/_2$EMTD (12.5 mg/kg) | 147.40±7.33* |
| EMTD (25 mg/kg) | 135.40±15.73* |
| 2EMTD (50 mg/kg) | 129.60±6.29* |
| Mexidol | |
| Control | 247.10±5.47 |
| $^1/_2$EMTD (12.5 mg/kg) | 235.30±13.30 |
| EMTD (25 mg/kg) | 209.10±6.07* |
| 2EMTD (50 mg/kg) | 232.70±7.14 |
| α-Lipoic acid | |
| Control | 236.50±5.49 |
| $^1/_2$EMTD (25 mg/kg) | 125.60±5.01* |
| EMTD (50 mg/kg) | 91.40±5.21* |
| 2EMTD (100 mg/kg) | 86.60±5.49* |

**Note.** *n*=10 in each group. Insulin coma was diagnosed by animal inability to change physiologically unnatural posture (hanging on a horizontal rod placed under the abdomen) for 60 sec [1]. *$p$<0.05 compared to the control (Mann–Whitney test).

shortening of the latency of insulin coma was produced by the maximum dose (2EMTD). It can be hypothesized that the maximum insulin-potentiating effect of 3-hydroxypyridine antioxidants in the dose equal to EMTD can be explained by the attained optimal balance between oxidative (NADPH-oxidase) transduction of the insulin signal and limited oxidative stress-dependent insulin resistance.

The insulin-potentiating effects of the test drugs were evaluated by their influence on GTT parameters (Table 2). Administration of the test drugs modulated glucose tolerance in experimental animals. Their effects were most pronounced 2 h after glucose load, when initial glycemia was followed by its minor decrease on the 60th minute of GTT, but blood glucose content remained above the pre-load level. Preliminary treatment with emoxipin and mexidol improved GTT parameters, which was seen from significant decrease of glycemia level 2 h after glucose load compared to the control values (Table 2). The most pronounced changes in this GTT parameter were observed after administration of mexidol, a derivative of both 3-hydroxypyridine and succinic acid. Mexidol in all tested doses reduced glycemia 2 h after glucose load. Emoxipin produced similar effect only in relatively high doses (EMTD and 2EMTD).

The pronounced insulin-potentiating effect of α-lipoic acid and reamberin did not provide their superiority over 3-hydroxypyridine derivatives in the effect on GTT. Moreover, α-lipoic acid was inferior to emoxipin and mexidol by the effect on glucose tolerance. The only dose of this drug ($^1/_2$EMTD) significantly reduced glycemia 60 min after glucose load. It cannot be excluded that α-lipoic acid only slightly increased glucose tolerance due to excessive potentiation of the autocrine effect of insulin on pancreatic β-cells [13]. This amplification of the negative feedback autocrine signal can inhibit secretion of endogenous insulin in response to glucose load. This mechanism can also underlie the decrease in hyperinsulinemia under the effect of rosiglitazone in states associated with insulin resistance [7] and paradoxical decrease in glucose tolerance under the effect of reamberin. This succinic acid derivative in all tested doses increased blood glucose concentration 2 h after glucose load. Injection of reamberin in a dose of $^1/_2$EMTD aggravated glycemia 60 min after glucose load. It should be noted that even preload values of all studied parameters increased 30 min after administration of reamberin in all the studied doses (Table 2). Qualitative differences between the effect of reamberin and other test drugs on GTT parameters can be explained by stimulating effect of

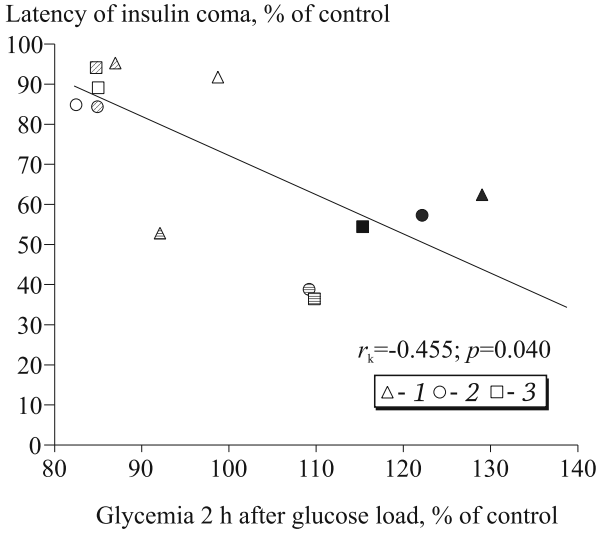


**Fig. 2.** Relationship between the effects of the test agents on latency of insulin coma and tolerance to glucose load. *1* — $^1/_2$EMTD; *2* — 1 EMTD; *3* — 2 EMTD. Light symbols: α-lipoic acid; dark symbols: reamberin; horizontal shading: mexidol; oblique shading: emoxipin.

**TABLE 2.** Effect of α-Lipoic Acid and 3-Hydroxypyridine and Succinic Acid Derivatives on Parameters of GTT Test (*M*±*m*)

| Group | GTT | | |
|---|---|---|---|
| | Glycemia before load, mmol/liter | Glycemia 1 h after load, mmol/liter | Glycemia 2 h after load, mmol/liter |
| Emoxipin | | | |
| Control | 4.91±0.33 | 9.95±0.50* | 8.97±0.39* |
| $^1/_2$EMTD (6.25 mg/kg) | 4.73±0.30 | 9.12±0.64 | 8.85±0.61 |
| EMTD (12.5 mg/kg) | 4.48±0.31 | 8.40±0.67 | 7.39±0.32** |
| 2EMTD (25 mg/kg) | 4.23±0.26 | 9.17±0.65 | 7.62±0.46** |
| Reamberin | | | |
| Control | 4.12±0.17 | 10.11±0.37* | 7.68±0.26+* |
| $^1/_2$EMTD (12.5 mg/kg) | 5.93±0.25** | 12.17±0.46** | 9.90±0.45** |
| EMTD (25 mg/kg) | 5.43±0.13** | 11.01±0.53 | 9.38±0.54** |
| 2EMTD (50 mg/kg) | 5.22±0.16** | 11.34±0.62 | 8.84±0.31** |
| Mexidol | | | |
| Control | 4.91±0.33 | 9.95±0.50* | 8.97±0.39* |
| $^1/_2$EMTD (12.5 mg/kg) | 4.41±0.24 | 8.75±0.75 | 7.79±0.69** |
| EMTD (25 mg/kg) | 5.19±0.34 | 8.89±0.65 | 7.61±0.51** |
| 2EMTD (50 mg/kg) | 5.06±0.49 | 9.11±0.73 | 7.60±0.63** |
| α-Lipoic acid | | | |
| Control | 4.12±0.17 | 10.11±0.37* | 7.68±0.26+* |
| $^1/_2$EMTD (25 mg/kg) | 4.20±0.32 | 8.79±0.26** | 7.07±0.26 |
| EMTD (50 mg/kg) | 3.85±0.38 | 10.07±0.77 | 8.38±0.47 |
| 2EMTD (100 mg/kg) | 3.71±0.27 | 10.44±0.48 | 8.42±0.41 |

**Note.** $n$=10 in each group. *$p$<0.05 compared to preload glycemia (only for control values, Wilcoxon test); +$p$<0.05 compared to glycemia 1 h after glucose load (only for control groups); **$p$<0.05 compared to the corresponding control values (Mann–Whitney test).

succinic acid on orphan receptor GPR91, which leads to activation of the rennin–angiotensin–aldosterone system [11] and can cause secondary activation of the sympathoadrenal system with a decrease in glucose tolerance.

Standardization of the obtained results by the mean values of the corresponding controls confirmed the assumption on opposite effects of the test drugs on insulin sensitivity and glucose tolerance (Fig. 2). The negative correlation between the effects of the test drugs on the latency of insulin coma and glycemia 2 h after glucose load was characteristic of all studied drugs. The revealed regularity can be related to opposite effects of "mild" prooxidant similar to α-lipoic acid on glucose consumption and glycogen synthesis in skeletal muscles [8]. It is known that these drugs enhance glucose consumption due to intensification of its intracellular oxidation and simultaneously suppress glycogen synthesis. This fact contradicts the commonly accepted opinion that α-lipoic acid is an insulinomimetic agent [8] despite its pronounced insulin-potentiating effect (Table 1). It should be emphasized that in contrast to α-lipoic acid, insulin limits oxidative stress and stimulates glycogen synthesis [9]. It seems likely that reamberin similar to α-lipoic acid by LPO-modulating (Fig. 1) and insulin-potentiating effects (Table 1) had an unfavorable effect on GTT due to the development of oxidative stress and concurrent suppression of glycogen synthesis.

It is known that antioxidants activate glycogen synthesis even in the absence of insulin [9]. It cannot be excluded that the favorable effect of 3-hydroxypyridine derivatives on GTT parameters is related to intensification of glucose deposition in the form of glycogen.

Our experiments demonstrated a relationship between LPO-modulating effect of the test drug *in vitro* and their effect on insulin sensitivity and glucose tolerance *in vivo*. The preparations producing prooxidant

effect *in vitro* (α-lipoic acid and reamberin) are characterized by pronounced insulin-potentiating activity, but only slightly increase (α-lipoic acid) or even decrease (reamberin) tolerance to glucose load. On the contrary, drugs with antioxidant activity (emoxipin and mexidol) increase glucose tolerance, but produce relatively weak insulin-potentiating effect.

The drugs studied here are used for the treatment of neuropathic complications of diabetes mellitus irrespective of its type and the prescribed glucose-lowering therapy [1,3,4]. Our findings suggest that the use of α-lipoic acid and reamberin in patients with diabetes mellitus receiving insulin is fraught with a risk of potentiation of its effects. At the same time, these preparations can be useful for correction of insulin resistance and hyperinsulinemia in patients with type 2 diabetes mellitus not receiving insulin. These results suggest that antineuropathic drugs with pro- and antioxidant activity should be prescribed differentially depending on the type of diabetes mellitus and individual need in insulin therapy. The pronounced increase in glucose tolerance under the effect of 3-hydroxypyridine derivatives together with their moderate insulin-potentiating activity puts a question on the preference of these drugs for the treatment of neuropathic complications of diabetes mellitus. This assumption is confirmed by clinically significant advantage of mexidol over α-lipoic acid by its ability to correct symptoms of distal symmetrical neuropathy and concomitant depression in patients with diabetes mellitus [1].

## REFERENCES

1. I. A. Volchegorskii, M. N. Alekseev, M. I. Volchegorskaya, and L. M. Rassohina, *Klin Med*., **86**, No. 10, 52-60 (2008).
2. I. A. Volchegorskii, I. I. Dolgushin, O. L. Kolesnikov, and V. E. Tseilikman, *Experimental Modeling and Laboratory Evaluation of Adaptive Reactions of the Organism* [in Russian], Chelyabinsk (2000),
3. I. A. Volchegorskii, M. G. Moskvicheva, and E. N. Chashchina, *Klin Med*., No. 11, 31-35 (2004).
4. I. A. Volchegorskii, M. G. Moskvicheva, and E. N. Chashchina, *Ter. Arkhiv*, **77**, No. 10, 10-15 (2005).
5. G. I. Klebanov, O. B. Lyubitskii, O. V. Vasil'eva*, et al., Vopr. Med. Khimii*, No. 3, 288-300 (2001).
6. E. B. Men'shikova, V. Z. Lankin, and N. K. Zenkov, *et al., Oxidative Stress, Prooxidants and Antioxidants* [in Russian], Moscow (2006).
7. F. Abbasi, Y. D. Chen, H. M. Farin, *et al., Am. J. Cardiol*., **102**, No. 1, 64-69 (2008).
8. N. Dicter, Z. Madar, and O. Tirosh, *J. Nutr.*, **132**, No. 10, 3001-3006 (2002).
9. J. L. Evans, B. A. Maddux, and I. D. Goldfine, *Antioxid. Redox. Signal.,* **7**, Nos. 7-8, 1040-1052 (2005).
10. B. J. Goldstein, K. Mahadev, X. Wu, *et al., Antioxid. Redox. Signal.,* **7**, Nos. 7-8, 1021-1031 (2005).
11. W. He, F. J. Miao, D. C. Lin, *et al., Nature*, **429**, 188-193 (2004).
12. D. Konrad, *Antioxid. Redox. Signal.,* **7**, Nos. 7-8, 1032-1039 (2005).
13. K. Mahadev, H. Motoshima, X. Wu, *et al., Mol. Cell. Biol.*, **24**, No. 5, 1844-1854 (2004).
14. Z. I. Storozheva, A. T. Proshin, V. V. Sherstnev, *et al., BMC Pharmacol.,* **8**, No. 1, 1-13 (2008).
15. G. I. Uwaifo, S. J. Parikh, M. Keil, *et al., Clin. Endocrinol. Metab.* **87**, No. 6, 2899-2905 (2002).