8. Molecular mechanisms of homocysteine toxicity and possible protection against hyperhomocysteinemia

Alexander Boldyrev
Research Center of neurology, Russian Academy of Medical Sciences and M.V. Lomonosov Moscow state University, Moscow, Russia

Abstract. Homocysteine is a naturally occurring intermediate connecting metabolism of methionine and cysteine and, subsequently glutathione. Its normal level in mammalian blood does not exceed 10 µmol/l, whereas vitamin deficient diet, increased stationary levels of reactive oxygen species, as well as neurodegenerative and cardiovascular diseases are usually accompanied by an increased content of homocysteine in peripheral blood. An increase in homocysteine levels above 50 µmol/l may be a risk factor for recurrent heart attacks and that above 150-200 µmol/l is a cause for ischaemic stroke. Persistent hyperhomocysteinemia with homocysteine levels of 300 µmol/l or higher may induce mental deficiency. Molecular mechanisms of homocysteine toxicity are not fully understood as yet. In the review, novel data are presented which demonstrate that homocysteine and homocysteic acid, a product of its spontaneous oxidation, induce activation of NMDA receptors in the brain neurons accompanied by an increase in the levels of the reactive oxygen species and resulting in induction of apoptotic and...
(under long-term stimulation) necrotic cell death. Recent findings showed that NMDA receptors are encountered not only in neuronal cells but also in megakaryocytes, red blood cells, lymphocytes, neutrophils, and cardiomyocytes. Thus, the number of target cells of homocysteine is sufficiently increased, thereby underlying the importance of developing new strategies to protect the body from hyperhomocysteinemia. In a model of prenatal hyperhomocysteinemia in rats, we have demonstrated that the neuropeptide carnosine (a natural antioxidant and immune modulator) protects animals against homocysteine toxicity with no change in the blood homocysteine levels. A suggestion was made that carnosine interferes with NMDA receptors preventing their excitotoxicity.

Introduction

1. Homocysteine as a risk factor for cardiovascular and neurodegenerative diseases

The total level of homocysteine (HC) in blood plasma of healthy donors amounts to 10-12 µmol/l and slightly increases with ageing [1]. Before the pubertal period, the HC content in blood of children is about 5 µmol/l (irrespective of the gender). Later on, this value increases to 6-7 µmol/l, being slightly lower in girls than in boys. In blood of adults HC levels reach 10-12 µmol/l, being slightly higher in men than in women [2]. An increase in HC blood levels with age is usually accompanied and followed by a decrease in the renal function with higher accumulation of HC in men than in women, depending on specificity of hormonal metabolism.

HC easily involves in red/ox reactions and its spontaneous oxidation results in homocysteic acid (HCA) accumulation [1] so that in the blood stream it exists both in its oxidized form (homocysteic acid, HCA) and in a complex with cysteine or proteins, with its bound form prevailing (accounting for over 70 %). The term “total HC” corresponds to all constituents including several homocysteine derivatives, and protein bound HC.

Excess of HC is considered to be a risk factor for a number of pathologies. The term “hyperhomocysteinemia” is used when the HC content exceeds 15 µmol/l. A level of 15-30 µmol/l corresponds to mild, 30-100 µmole/l – to moderate, and more than 100 µmol/l – to severe hyperhomocysteinemia [1, 3]. Common causes of hyperhomocysteinemia typically include: renal impairment induced by several pathologies [4], deficiency of vitamins involved in HC metabolism [5, 6], overloading with dietary methionine or excessive accumulation of reactive oxygen species (ROS) in tissues [7] as well as deficiency of enzymes, which promote HC metabolism (see below). In some cases, normalization of blood HC levels may be achieved by diet modulations. Adherents to dietary restrictions believe that one of the positive effects of caloric restriction prolonging the life span is a decrease in methionine supply [8].
Apparent disposition to hyperhomocysteinemia is encountered in smokers [9] or cafe amateurs [10]. Regular alcohol consumption reduces folate and vitamin B\textsubscript{12} status and increases HC levels in blood of healthy donors [11].

In the second half of the XX century, homocysteinuria syndrome was described as related to cystationine-β-synthase deficiency [12]. Homocysteinuria is usually accompanied by thromboembolia, progressive cardiovascular diseases and mental deficiency [13]. Moreover, an increase in blood HC was demonstrated to induce atherosclerosis because HC stimulates an atherogenic action of cholesterol [12, 13]. Thus, hyperhomocysteinemia is one of the pathogenic factors of atherosclerosis [14, 15]. It also provokes myocardial infarction [16], cerebral stroke [17], and complicates diabetes [18]. Severe hyperhomocysteinemia was shown to result in brain convulsions and dementia [19], as well as may contribute to Alzheimer’s disease (AD) [20]. Recent studies showed that genetically provided deficit of methylenetetrahydrofolate reductase and coherent accumulation of HC in blood is a risk factor of AD in human population [21].

In AD patients, plasma and brain HC levels were found to be increased significantly [22, 23]; the same was noted for the cerebrospinal fluid [24]. Statistical analysis showed that with plasma HC levels above 14 μmol/l there is a two-to-five-fold increase in the risk factor of AD as compared with that in the age-matched controls [25, 26]. Therefore, hyperhomocysteinemia is often considered as a predictive factor for cognitive impairment in the elderly [24, 27] and for AD [28], although there is debate of whether hyperhomocysteinemia is a risk factor for, or a risk marker of AD [29].

HC may also play a provocative role in neurotoxicity of amyloid-β peptides, and the presence of amyloid-β in the brain of AD patients was shown to be exacerbated by HCA [30]. Both HC and HCA may increase sensitivity to toxic effects of amyloid-β peptides directly affecting their conformation and inducing the formation of neurotoxic β-fibrils [30, 31].

Currently, it is widely accepted that HC is a risk factor for cardiovascular and neurodegenerative diseases [7, 21, 22], although the molecular grounds of homocysteine toxicity are not fully understood. The present review is devoted to analysis of molecular mechanisms of HC toxicity.

2. Neurotoxic effects of homocysteine

A toxic effect of HC and its derivatives on the cerebellum granule cells was described about a decade ago and it was suggested to be realized via glutamate receptors [32-34]. The most expected candidate could be glutamate receptors activated by N-methyl-D-aspartic acid (NMDA), the so-called
NMDA receptors. Their activation results in a significant rise in intracellular Ca ions and severe accumulation of ROS inside the neurons [35]. An increase in ROS levels could be suggested as an immediate reason of the toxic effect, for both superoxide dismutase and catalase proved to be strongly protective [32].

Moreover, the neurotoxic effect of HC was prevented by both MK-801 (an irreversible non-competitive antagonist of NMDA-receptors) and α-methylcarboxyphenylglycine, which is known to inhibit metabotropic Group I glutamate receptors, whose effect is associated with an activation of phospholipase A2 and phosphatidyl inositol-3-phosphate induced mobilization of intracellular calcium ions from the sarcoplasmic reticulum [36]. It was found that another antagonist of Group I metabotropic receptors, LY 367385, which also partially protects neurons from toxic effect of HC could completely prevent neuronal death induced by HC, once added in a combination with MK-801, whereas agonist of metabotropic Group I receptors, t-ADA induced neurodegeneration [37]. Thus it was concluded that both ionotropic and metabotropic glutamate receptors are involved in neurotoxic effects of HC.

It was found recently that one of the consequences of incubation of hippocampal slices with HC is inactivation of intracellular protein phosphatases and subsequent hyperphosphorylation of neurofilaments which resulted in cytoskeleton impairments [38]. This phenomenon was found to be an important cause of damaging the neuronal membrane.

Nevertheless, HC should be considered rather a weak neurotoxin. In the in vitro experiments, its cytotoxic effect appears at concentrations higher than 1 mmol/l and induces cellular necrosis [37]. A similar neurotoxic action may be achieved by glutamate at only 2 times lower concentration [32]. Some HC derivatives, like HCA, which is considered to be an endogenic neurotoxin [39] may have a much stronger neurotoxic effect than HC [40, 41]. Sensitivity of neurons to HCA is sufficiently higher than that to HC and at 10-100 µmol/l concentration (moderate hyperhomocysteinemia which is characteristic of AD patients), HCA activates NMDA receptors [42, 43, 44].

HC and HCA render a similar effect on an increase in calcium and ROS levels inside the neurons, the latter being suppressed by both N-acetylcysteine (cell membrane penetrating antioxidant) and BAPTA-AM (cell membrane penetrating Ca-buffer) [45]. The authors concluded that HC or HCA induced a calcium signal preceding intraneuronal ROS accumulation. ROS signal rather quickly (after 1-3 hrs) induces externalization of phosphatidylserine on the neuronal membrane [36, 44], which corresponds to initiation of the apoptotic process.
Homocysteine as a risk factor for neurodegeneration and AD

It was demonstrated that an intraventricular injection of either NMDA or HCA to two-week-old rats induced long-lasting convulsions accompanied by massive apoptotic death of neurons in several regions of the brain; the NMDA receptor antagonists act as anticonvulsants [43].

Thus, HC and HCA induce an excitotoxic effect influencing preferably the NMDA receptors, which is easy to explain in terms of similarity of their structures:

![Chemical structures of Glutamate, NMDA, Homocysteine, and Homocysteic acid]

3. Discovery of NMDA-receptors in the non-neuronal cells

It is clear now that glutamate receptors diversely spread in several tissues. Metabotropic glutamate receptors associated with G-proteins present in various cells and even ionotropic glutamate receptors belong not only to the brain. Several years ago, NMDA receptors were described in rodent and human lymphocytes [46, 47] and it was found that their activation with NMDA induces calcium [48] and ROS [47, 49] accumulation.

Biological significance of these receptors in immune competent cells is not fully understood but the levels of their expression were found to depend on the functional state of the cells. Incubation of lymphocytes \textit{in vitro} with phytohaemagglutinin (lymphocyte activator of plant origin) rapidly increases the portion of cells, which express NMDA receptors [49, 50]. Under \textit{in vivo} conditions, inflammatory factors also increase the number of lymphocytes expressing these receptors [51].

Simultaneous presence of ionotrophic and metabotropic receptors on the membrane of activated lymphocytes makes these cells close to neuronal cells where interaction between receptors of these classes is an intrinsic mechanism of regulation of cellular function [52]. It is possible that the
interaction between metabotropic and ionotropic receptors regulates efficiency of the cytokine synthesis. Such a conclusion was made when γ-interferon accumulation was measured by IL-2 activated lymphocytes expressing both ionotropic (NMDA activated and AMPA activated) and metabotropic Group I glutamate receptors [47]. It was found that neither NMDA, nor glutamate affect γ-interferon accumulation in the native (non-activated) cells and the level of the cytokine synthesis is neglected. Once incubated with IL-2 the cells start to accumulate γ-interferon, and this process is inhibited in the presence of NMDA but stimulated in the presence of glutamate [49]. The authors interpreted these data in such a way that ionotropic receptors of NMDA class suppress and metabotropic Group I receptors stimulate the cytokine production [49]. Thus, glutamate may play a role of both a neuromediator (in the neuronal system) and immunomediator (in the immune system) [53].

Recently, a similar situation was described in other immune cells of rodents possessing phagocytic properties, neutrophils. Neutrophils isolated from peripheral blood of intact animals were free from NMDA receptors, whereas they have a number of adenosine receptors regulating their immune function [54]. Adenosine receptors of A1 class affect phagocytosis and those of A3 class affect degranulation, whereas A2 adenosine receptors control the cAMP levels, thus regulating neutrophic activation [54-56].

Hence, the result of activation of adenosine receptors of the neutrophils would depend on the species of receptors involved in realization of the signal. For example, an interaction between A1 or A2 adenosine receptors with Fcγ receptor is described in the literature [56]. Activation of A1-receptors switches on Fcγ receptors and results in ROS generation, whereas activation of A2 receptors switches off Fcγ receptors and suppresses ROS accumulation [57]. Interrelations between A1 receptors and NMDA receptors are also noted on the postsynaptic membrane of the neuronal junction where activation of A1 suppresses the NMDA dependent ionic fluxes [58]. At the same time, on the presynaptic membrane, adenosine suppresses the glutamate release in the synaptic cleft, whereas glutamate via activation of NMDA receptors stimulates adenosine release in synaptic cleft [59]. Revealing NMDA receptors in lymphocytes [47-49] suggests the presence of similar mechanisms of interaction between the glutamate and adenosine receptors in these cells.

We have found recently that lymphocytes and neutrophils isolated from an inflammatory region of rats expressed NMDA receptors on their membrane contrary to the intact cells which are predominantly free from them [51, 60]. The functional significance of this phenomenon is still obscure but one can suggest that they may regulate ROS production during cell
Homocysteine as a risk factor for neurodegeneration and AD

133

activation. Especially important is that HC can stimulate ROS production in these cells presumably through NMDA receptors [60, 61]. With this in mind, identification of NMDA receptors in megakaryocytic cells (platelet precursors) [62, 63] and in cardiomyocytes [64] where they can regulate Ca-fluxes across the outer membrane is very noticeable. Thus, we conclude that NMDA receptors are involved in intracellular signaling not only in the brain but also in a broad variety of other tissues.

4. Effect of homocysteine on red and white blood cells

The above data concerning the capability of the immune competent cells to express NMDA receptors makes it interesting to evaluate the effect of HC and HCA on their function. Recently, we have analyzed the effect of HC and HCA on both cytokine producing cells (lymphocytes) and phagocytic cells (neutrophils). It was found that in lymphocytes, these receptors are similar to those in neurons by their ability to increase calcium and ROS accumulation [36, 46, 47] and by the effects of HC and HCA [65]. Effective concentrations of HC are similar to those found in moderate and severe hyperhomocysteinemia (100-500 \(\mu\text{mol/l}\)) and effects are quickly manifested: even a 1-to-3-hour incubation is enough to demonstrate the initial steps of cellular death via apoptosis or necrosis.

As we noted earlier, neutrophils isolated from peripheral blood of intact animals are practically free from the functionally active NMDA receptors and HC does not affect the ROS levels. It can however stimulate ROS production when the cells are stimulated by fMet-Leu-Phe (fMLP), which itself brings about the so-called “respiratory burst”. This phenomenon is known to depend on specific fMLP receptors [66], which are unlikely to interact with HC (HC does not affect the neutrophil function in the absence of fMLP) and modulation of the respiratory burst by HC requires its interaction with other receptors.

The role of HC target may be played by adenosine receptors [67] virtually interacting with HC, thus resulting in stimulation of ROS production [61, 68]. Inhibitory analysis showed that A2 receptors are involved in HC effect resulting in over-production of ROS by neutrophils [61]. Interestingly, HCA renders a weaker effect on neutrophils than HC does, whereas it affects neuronal NMDA receptors more effectively than HC. Thus, adenosine receptors demonstrate higher affinity to HCA whereas NMDA receptors demonstrate higher affinity to HC.

An ability of neutrophils to express NMDA receptors during activation by inflammatory cytokines suggests involvement of these receptors in ROS production by these cells. Actually, HC stimulates the respiratory burst of
such neutrophils and this effect is sensitive to MK-801 [60]. Thus, HC
hyperactivates the NMDA receptors of both the neuronal and immune
competent cells. It shows that hyperhomocysteinemia is a factor exhausting
the functional activity of both the neuronal and immune systems.

Recently, a strong hemolytic effect of HCA was described consisting in
acceleration of red blood cells hemolysis induced by several unfavourable
factors [69]. Preincubation of RBC with HCA increased the rate of acidic
hemolysis and decreased the lag-period. Such action can be easily
understood, taking into consideration possible presence of NMDA receptors
on the erythrocytic membrane [69, 70]. Their activation can induce the Ca-
entry into red blood cells and modify their functional properties.

A possible toxic effect of HC on the platelets is also noteworthy. A high
blood level of HC stimulates platelets aggregation, which may be
associated with increased ROS production and damage of vascular
endothelial cells [71, 72]. These effects at least partially can be a result of
HC effect on NMDA receptors recently described in megacaryocytes
[62, 63].

5. Possibilities to protect the body from toxic effects of
homocysteine

As we discussed, impaired HC metabolism results in its accumulation in
blood stream and this accompanies a number of pathologies [7, 73].
High levels of blood HC may be not an immediate cause of a pathological
state but their consequences. In any case, a toxic action of HC will
aggravate the development of disease revealing neurological symptoms. Thus,
hyperhomocysteinemia is associated with serious complications, impelling to
the search of protectors against HC toxicity.

An important aspect of hyperhomocysteinemia consists in toxic effects of
HC on the female body in pregnancy. HC easily penetrates from the maternal
blood into the fetus to render a teratogenic/fetotoxic action. HC levels in the
maternal blood were found to have a negative correlation with the body
weight of the newly-born infant [74]. Hence, all the above-described toxic
effects of HC may induce pregnancy complications [75, 76].

A commonly accepted means to compensate hyperhomocysteinemia is a
systemic increase in dietary vitamins (B₆, B₁₂, folic acid). In some cases,
however, especially when an increase in HC levels is induced by genetically
provided deficiency of enzymes of its metabolism dietary regulation is not
sufficient. That is why it is important to devise a special strategy to defend
the body from HC toxicity. Because accumulation of ROS and misbalance in
cell signaling in NMDA receptor’s expressing cells is one of the features of
Homocysteine as a risk factor for neurodegeneration and AD

HC toxicity administration of drugs modulating properties of the receptors may have undesirable side effects. On the other hand, the use of natural metabolites able to regulate the intracellular ROS levels and preserve viability and functional activity of the cells could be effective.

In order to develop such approach we turned the attention to neuropeptide carnosine (β-alanyl-L-histidine) which is a specific constituent of excitable tissues being able to protect the brain neurons against oxidative stress [77, 78]. Carnosine prevents neuronal death induced by excitotoxic effects of NMDA in vitro [79, 80]. It is characterized by extremely low toxicity [81, 82] and ability to penetrate the blood-brain barrier after administration into blood stream [83]. Excess of carnosine is quickly cleaved by serum carnosinase preventing effects of possible over-loading [84, 85]. Carnosine was also found to protect the brain under stroke conditions in vivo [86, 87]. The described recently favourable effect of carnosine on red blood cells under acidic hemolysis aggravated by HCA [69] suggested that this compound possesses a diversity of properties besides its antioxidant activities.

In order to estimate possible protection of the body from toxic effects of HC, we used a recently developed model of prenatal hyperhomocysteinemia induced by over-loading of pregnant rats with excess of methionine (1g/kg body weight daily) [88, 89]. Thus the prenatal development of the brain is implemented under stable hyperhomocysteinemia (in our case, blood HC levels increased from 5.9 ± 1.8 to 33.0 ± 3.9 µmol/l) [89], resulting in a decrease in the average number of neonatal rats in the litter and a marked decrease in their body weight (Table 1).

Table 1. Characteristic of pups in the progeny of several groups of rats (p1 estimates the statistically significant difference from group 1, and p2 – that from group 2), from [90] with permission.

<table>
<thead>
<tr>
<th>Groups of animals</th>
<th>Number of families</th>
<th>Average number of newborns in the litter</th>
<th>Weight, g (10 days old)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (intact animals)</td>
<td>6</td>
<td>12 ± 2</td>
<td>23.3±0.4 p2&lt;0.05</td>
</tr>
<tr>
<td>Group 2 (methionine administration)</td>
<td>4</td>
<td>7 ± 1 p1&lt;0.05</td>
<td>18.9±0.5</td>
</tr>
<tr>
<td>Group 3 (methionine + carnosine .</td>
<td>6</td>
<td>13 ± 2 p1&gt;0.05</td>
<td>24.1±0.6 p2&lt;0.05</td>
</tr>
<tr>
<td>administration)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Animals whose prenatal and early postnatal development was under stable hyperhomocysteinemia were tested using the Morris’ water test [91]. An efficiency of searching for the platform in a water pool was estimated after preliminary training. The test demonstrated that the animals in the methionine-treated group were characterized by substantially poorer memory (Table 2). The period of search the platform in the water pool for these animals was several times longer, the swimming velocity was somehow less (not statistically significant), and duration of search in the central area of the pool (where the platform was located) amounted to only 7% of the total time (in the control group, this time was 20% of the total search period). Moreover, analysis of the cerebellum neurons of the animals showed desensitization of the NMDA receptors to NMDA, HC or HCA [90].

The group-three animals treated with carnosine (taken with drinking water at a daily dose of 100 mg/kg bogy mass) simultaneously with methionione substantially differed from group-two animals and by some parameters were similar to the control group. As can be seen from Table 1, pregnancy was more successful and the number of neonates in the litter, as well as their weight was similar to that for intact animals. Moreover, they were more successful in the learning test (Table 2). Finally, suspension of the cerebellum neurons isolated from these animals, revealed the lowest percent of dead cells found and the mean fluorescence of viable neurons (reflecting the intracellular ROS levels) was lower than that of the neurons from the cerebellum of the group-two animals.

All these data demonstrated that systemic administration of carnosine turned out protective against HC toxicity and had improved the conditions for the foetus development in spite of a similar high content of HC in the blood.

Table 2. Learning of the animals of 3 groups (designed as in Table 1) obtained from Morris’ water test (from [90] with permission).

<table>
<thead>
<tr>
<th>Parameter registered</th>
<th>Group 1 (n=18)</th>
<th>Group 2 (n=18)</th>
<th>Group 3 (n=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time to reaching the platform, s</td>
<td>20 ± 7</td>
<td>140 ± 18</td>
<td>45 ± 6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p1&lt;0.01</td>
<td>p2&lt;0.01</td>
</tr>
<tr>
<td>Average rate of swim, m/s</td>
<td>0.24 ± 0.02</td>
<td>0.18 ± 0.02</td>
<td>0.25 ± 0.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p2&gt;0.05</td>
<td></td>
</tr>
<tr>
<td>Duration of swimming in the central area of the pool (% of the total period)</td>
<td>20 ± 7</td>
<td>7 ± 5</td>
<td>35 ± 5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p1&lt;0.01</td>
<td>p2&lt;0.01</td>
</tr>
</tbody>
</table>
Homocysteine as a risk factor for neurodegeneration and AD

(because carnosine did not decrease the blood HC levels) [90]. Consequently, a protecting effect of carnosine manifested itself not in metabolism of homocysteine but in suppression of its toxic action. It is not clear so far, how carnosine acts in the in vivo conditions – does it suppress affinity of NMDA receptors to HC, prevent accumulation of ROS, or use other (still unknown) mechanisms of protection?

6. Carnosine improves therapeutic outcomes in patients with neurodegenerative diseases

All the data characterizing efficiency of carnosine as a natural brain protector in animals led us to a conclusion that it might be appropriate to use it for treatment of neurodegenerative diseases in man. At the end of this Chapter, the first experience is presented related to this new approach.

Discirculatory encephalopathy (DE) is characteristic of chronic sub-acute expansion accompanied by increased levels of blood HC [17]. A double-blind placebo-controlled trial comprised a total of 42 patients (both men and women) diagnosed with chronic DE [92]. Carnosine was included into basic (symptomatic) therapy, given at two doses (either 0.75 or 2.0 g, daily), with the treatment duration amounting to 21 days. At the end of the trial, some neurochemical and neurological characteristics of the patients before and after the treatment were compared. The basic therapy, as well as its combination with low doses of carnosine turned out practically ineffective in improvement of either the somatic or psychological state of the patients. However, a combination of the basic therapy with carnosine taken at a higher dose (2.0 g per day) was characterized by noticeable effects. Resistance of blood lipoproteins to Fe^{2+}-induced oxidation increased and the rate of acidic hemolysis of red blood cells decreased. At the same time, the ability of leucocytes to generate (in vitro) the zimozane-induced respiratory burst was significantly accelerated. All these data demonstrated that carnosine improved the antioxidant defence system, as well as the ability of the immune system to withstand extrinsic factors.

A conclusion on the cognitive function of the brain was made based on analysis of the induced potentials P300 from the electroencephalogram of patients. Sufficient dynamics of P300 potentials was only noted in the basic therapy patients treated with a high dose (2.0 g per day) of carnosine. Only in this case (not in the case of basic therapy alone or in its combination with low doses of carnosine) the latency of P300 peak decreased from 378 ± 21 msec before treatment to 345 ± 12 msec after treatment ($p < 0.05$) and the number of low-amplitude spikes fell from 60% to 27% ($p < 0.01$). This demonstrated a positive effect of carnosine on the brain cognitive function of DE patients.
It is noteworthy that such an effect of carnosine was also demonstrated in spite of pronounced hyperhomocysteinemia in patients. This again supports a point of view that the carnosine’s effect is based on prevention of HC toxicity rather than on its metabolic neutralization.

**Parkinson’s disease.** Parkinson’s disease (PD) is a chronic neurodegenerative disease characterized by progressive loss of dopaminergic neurons in the *substantia nigra pars compacta*. The exact aetiology of PD remains obscure, but what we clearly know is that the disease is of a multifactorial nature, including both environmental and genetic factors, which result in development of oxidative stress in specific areas of the brain. One of the factors accompanying PD development is pronounced hyperhomocysteinemia [93].

A conventional protocol of PD treatment consists of replacing lost dopamine with its agonists including L-DOPA therapy, administration of MAO B or catechol-0-methyltransferase inhibitors, and other factors aimed at facilitating symptomatic improvement, not altering the course of disease. For this reason, treatment of PD patients is accompanied by several side effects that render the treatment nearly ineffective [94]. Therefore, it is important to develop complex treatment of PD including in the protocol antioxidants and/or neuroprotectors [93, 94].

Several recent publications illustrate a positive effect of carnosine used as an additive to basic therapy of PD patients [95, 96]. A pilot clinical trial was composed of 36 patients with trembling-rigid and trembling manifestations of PD to be compared with 20 apparently healthy donors comprising a control group. Basic therapy consisted of DOPA-containing drugs *Madopar* or *Nacom*, dopamine receptor agonists *Pronoran* or *Mirapex* and uncompetitive antagonist of NMDA receptors, amantadine at individually selected doses depending on the state and severity of clinical manifestations. For half of the PD patients carnosine was prescribed at a daily dose of 1.5 g. The treatment lasted for 30 days and preliminary data are presented below.

After basic treatment, the baseline level of neurological symptomatic of patients decreased from 38.9 ± 2.5 to 32.5 ± 2.0 points (measured by the Unified Parkinson’s Disease Rating Scale, UPDRS) which corresponded to a 16.4% improvement. Combining basic treatment with carnosine decreased the symptomatology to 24.9 ± 2.1 (36% improvement), which was at least 2 times better. Thus, carnosine included in the protocol of treatment as an additional component significantly improved the neurological state of the patients.

In the carnosine-treated group, an improvement of the locomotor system (rigidity of extremities, and upper-limb movements) amounted to 32 – 38% (p < 0.05) compared with the basic-therapy group which correlated well with improvement of one of the most important clinical signs of parkinsonism – hypokinesia. The authors noted that the so-called “every day activity” was also
improved significantly more in the carnosine-treated patients, which gave them ability for more independent self-service [96]. No any negative side effect was detected.

A decrease in the neurological symptomatology of PD patients correlated with a decrease in blood serum carbonyl levels and an increase in resistance to oxidation of lipoproteins in blood plasma, as well as in restoration of SOD activity (measured in red blood cells), with the increment of SOD restoration being in a correlation ($r = 0.654$) with a neurological symptomatic decrement. The authors concluded that a combination of carnosine with basic therapy of PD patients may be a reasonable way to improve the PD treatment and to decrease possible toxic effects of over-loading of DOPA containing drugs.

**Alzheimer’s Disease.** Alzheimer’s disease is another example of a neurodegenerative disorder complicated by an oxidative stress and increased levels of HC in blood of patients [22, 27, 28]. In the available literature we found no information concerning carnosine used to treat AD patients; however, based on the above data such an approach seems very reasonable.

### 7. Conclusion

In conclusion, hyperhomocysteinemia is one of serious risk factors for cardiovascular and neurodegenerative diseases, as well as other pathologies in which various manifestations of oxidative stress are involved. We have shown that the molecular basis for toxicity of homocysteine and its derivatives consists in over-activation of the NMDA class specific glutamate receptors. A wide distribution of these receptors in a number of tissues including the brain, heart, and immune competent cells makes HC toxic for many tissues. Successful attempts to use naturally occurring compounds to protect the body from HC toxicity might find a favourable application in present-day clinical protocols.

I am thankful to Academician Z.A. Suslina, Professor S.N. Illarioshkin and Professor I.A. Ivanova-Smolenskaya (Research Center on neurology, Moscow, Russia) for organization of clinical trials and Drs. B.A. Kistenev, M.Yu. Maximova, V.V. Gnezditsky, G.Ch. Bagyeva and M.A. Loskutnikov for highly professional participation in clinical trials.

The work is supported by RFBF Grant #06-04-49675 (Russia).

### References

44. Boldyrev, A.A. 2006, Neurochemistry (Moscow), 23, 165.
51. Mashkina, A.P. - personal communication.
60. Bryushkova, E.A. - personal communication.
70. Makhro, A.V. - personal communication.
Neurosci., 25, 133.
89. Makhro, A., Mashkina, A., Solenaya, O., Trunova, O., Kozina L., Arutyunian, A.
90. Makhro, A., Mashkina, A., Solenaya, O., Trunova O., Tyulina, O., Bulygina, E.,
91. Steele, R.J., and Morris, R.G. 1999, Hippocampus, 9, 118.
92. Fedorova, T.N., Belyaev, M.S., Trunova, O.A., Gnezditsky, V.V., Maximova,
93. Duan, W., Ladenheim, B., Cutler, R.G., Krum, I.I., Cadet, J.L., and Mattson,
94. Youdim, M, Geldenhuys, W., and van der Schyf, C. 2007, Parkinsonism and
Related Disorders, 13, S281.
95. Boldyrev, A., Fedorova, T., Stvolinsky, S., Stepanova, M., Dobrotvorskaya, I.,
Kozlova, E., Bagyeva, G., Ivanova-Smolenskaya, I., Markova, E., and
96. Boldyrev, A., Fedorova, T., Stepanova, M., Dobrotvorskaya, I., Kozlova, E.,
Boldanova, N., Bagyeva, G., Ivanova-Smolenskaya, I., and Illarioshkin, S. 2008,
Rejuv. Res., 11 (8), 988.