

The Role of Hyperhomocysteinemia in Aged Rats

Cristina-Sorina Cătană¹ and Gabriel Samasca^{2*}

¹Department of Medical Biochemistry, Iuliu Hatieganu, University of Medicine and Pharmacy, Cluj-Napoca, Romania

²Medical Immunology Department, Iuliu Hatieganu, University of Medicine and Pharmacy, Cluj-Napoca, Romania

Introduction

In humans, HHcy causes various symptoms such as mental retardation, epilepsy, epileptic seizures and atherosclerosis, whose pathology is not fully understood. Pro-inflammatory mediators released by activated microglia secrete various neurotoxic factors such as NO and ROS, which contribute to neuronal degeneration in neurodegenerative diseases [1,2]. Within this context, HHcy proved to cause neuronal death, frequently associated with increased ROS levels [3]. Chronic treatment with Hcy induces oxidative stress in the rat brain by increasing lipid peroxidation and decreasing enzymatic and non-enzymatic antioxidant protection [4]. The reference range for mean plasma Hcy values in rats is situated between $10.4 \pm 0.6 \mu\text{M}$ [5].

Oxidative stress is the main etiopathogenetic mechanism involved in HHcy induced changes. Hcy levels increase with age while antioxidant protection decreases [6]. In the presence of molecular oxygen, Hcy causes intra- and extracellular auto-oxidation and generates ROS, which, in their turn, react with cell constituents and small molecules such as NO. Hcy administered to cell cultures reduces NO bioavailability and causes hydrogen peroxide-mediated cytotoxicity. Specific molecular targets are an alternative to the hypothesis of oxidative stress, as Hcy either interacts with them or models their extra- or intracellular activity [7].

SOD activity proved to increase in a dose-dependent manner after rat aortic smooth muscle cells were treated with D, L-Hcy (0-500 $\mu\text{mol/L}$); however, no effect on catalase activity was noticed [8]. The negative effect of Hcy is also due to homocysteinylated, which alters the biological function of several enzymes, receptors, growth factors and structural proteins [9]. The formation of homocysteinylated protein derivatives depends on exposure duration and Hcy concentration [10].

The short exposure period (two hours) could decrease SOD activity [4]. Previous studies indicated that Hcy administration greatly reduced copper status in rats. The level of erythrocyte SOD, a copper-dependent enzyme, decreased in rats treated with Hcy compared with untreated rats [11].

In humans, Hcy levels increase with age. This phenomenon is partially explained by gradual kidney function impairment. The increase is obvious after the age of 60. A two-fold increase in Hcy levels usually occurs throughout a person's lifetime [12].

After the age of 60, the increase is much more rapid (1 $\mu\text{mol/L}$ every 10 years). Gender differences have also been noticed, higher levels being registered in men (approximately 2 $\mu\text{mol/L}$) compared with women. Still, gender differences become less visible with age, the percentage of male and female subjects with Hcy levels above the RR being similar [13].

Acute Hcy administration significantly decreased IL-6 levels in older rats ($p < 0.008$) compared with controls. In young animals, a statistically insignificant increase ($p < 0.3$) was recorded after Hcy administration.

Serum IL-6 levels increase with age. In this context, a significant difference was observed between 1-month-old and 12-months-old rats belonging to control groups I and III. This result explains the pro-inflammatory status present in aging as well as in the neurodegenerative

diseases associated with it [14,15]. The short exposure (two hours) to an increased Hcy concentration decreased IL-6 levels but only in old rats (groups III and IV) by a mechanism that is yet to be deciphered.

In young rats, IL-6 levels displayed a statistically insignificant increase after Hcy administration. Several studies focused on the relation between Hcy and inflammation. The pro-inflammatory status in aging proved to be associated with HHcy [16]. Furthermore, levels of acute phase proteins such as fibrinogen, CRP and α -1-antichymotrypsin correlate with circulating Hcy levels [14,17].

As previously mentioned, IL-6 can interact with the vitamin B₆ metabolism and compromise the activity of cystathionine β -synthase, which is involved in Hcy catabolism, thereby rising plasma Hcy levels [18]. Interestingly, pro-inflammatory cytokine levels are associated with HHcy-related pathology such as ischemic stroke, myocardial infarction and more recently, osteoporosis. These manifestations were reported independently of dietary vitamin intake, circulating vitamin levels and cardiovascular risk factors [19-22].

HHcy increases the production of ROS by auto-oxidation and alteration of GPx-1 expression. The oxidative stress induced by Hcy has the following consequences:

- Oxidative inactivation of endothelium-derived NO[•] by hydrogen peroxide [23].
- Lipid peroxidation [24].
- Formation of lipid peroxynitrite [25].
- Uncoupling of eNOS (endothelial nitric oxide synthase) mediated by peroxynitrite and the oxidation of its cofactor tetrahydrobiopterin [26].

On the other hand, Hcy increases the expression of IL-1 β and decreases the vascular synthesis of NO[•] [27] through NMDA (N-methyl-D-aspartate) glutamate receptor activation in cultured rat endothelial or smooth muscle cells. IL-1 β markedly enhances GPx gene expression. IL-6 has similar yet weaker effects than those induced by IL-1 β [28].

It was observed that 5 mmol/L Hcy decreased steady-state mRNA for GPx by approximately 90% [29]. By inhibiting GPx activity, Hcy seems to be a major cause of neurodegenerative pathology typically linked with oxidative stress [30].

***Corresponding author:** Gabriel Samasca, Department of Immunology, Hatieganu, University of Medicine and Pharmacy Cluj-Napoca, Romania, E-mail: Gabriel.Samasca@umfcluj.ro

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Conclusions

There is a clear association among HHcy, pro-inflammatory status and oxidative stress as whole blood GPx and SOD activities depend on the aging process.

The inflammatory process produced by Hcy administration created oxidative stress.

GPx activity decreases in both age groups after Hcy administration. Hcy seems to be an effective pro-inflammatory messenger able to activate various cytokines by enhancing oxidative stress in young and old rats.

Erythrocyte SOD activity, an oxidative stress marker, decreased in young and old rats following Hcy administration even after a short exposure period. HHcy changed the antioxidant balance by generating ROS, the enzyme being the target of the excessively produced superoxide anion.

The aging process is directly related to oxidative stress and pro-inflammatory status. These conclusions could facilitate new therapeutic strategies in patients with neurodegenerative, cardiovascular, metabolic diseases associated with the aging process.

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