

Increased inflammatory markers in brain and blood of rats subjected to acute homocysteine administration

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Abstract Hyperhomocysteinemia plays an etiologic role in the pathogenesis of disorders, including homocystinuria and neurodegenerative and cardiovascular diseases. In the present study, we studied the effect of acute administration of homocysteine, similar to that found in homocystinuria, on parameters of inflammation such as cytokines (TNF- α , IL-1 β and IL-6), chemokine CCL2 (MCP-1), nitrite and acute phase-proteins (C-reactive protein and α_1 -Acid glycoprotein) levels in brain and blood of rats. In addition, a differential count of blood leukocytes was performed. Wistar rats, aged 29 days, received a single subcutaneous injection of saline (control) or homocysteine (0.6 $\mu\text{mol/g}$ body weight). Fifteen minutes, 1 h, 6 h or 12 h after the injection, the rats were sacrificed and serum, hippocampus and cerebral cortex were used. Results showed that homocysteine significantly increased proinflammatory cytokines (TNF- α , IL-1 β and IL-6) and chemokine CCL2 (MCP-1) in serum, hippocampus and cerebral cortex. Nitrite levels also increased in hippocampus and cerebral cortex at 15 min, 1 h and 6 h, but not 12 h after homocysteine administration. Acute phase-protein levels were not altered by homocysteine. The percentage of neutrophils and monocytes significantly increased in blood at 15 min and 1 h, but not at 6 h and 12 h after acute hyperhomocysteinemia, when compared to the control group. Our results showed that acute administration of homocysteine increased inflammatory parameters, suggesting that inflammation might be associated, at least in part,

with the neuronal and cardiovascular dysfunctions observed in homocystinuric patients.

Keywords Homocysteine · Cytokines · Chemokine · Acute-phase proteins and nitrite

Introduction

Elevated homocysteine (Hcy) levels can be found in several disorders, such as homocystinuria, and neurodegenerative and neuroinflammatory diseases (Mudd et al. 2001; Tyagi et al. 2009). Homocystinuria is an inborn error of metabolism caused by severe deficiency of cystathionine β -synthase (CBS, EC 4.2.1.22) activity. Affected patients present alterations in various organs and systems, especially the central nervous and the vascular systems and present manifestations that include mental retardation, seizures and atherosclerosis (Mudd et al. 2001), whose underlying mechanisms are still obscure.

Inflammation often elicits a generalized sequence of events, known as the acute phase response (Suffredini et al. 1999), which is mediated by the generation of early response cytokines, such as interleukin (IL)-1 β , IL-6 and tumor necrosis factor-alpha (TNF- α), acute-phase proteins, expression of cell-surface adhesion molecules, as well as chemotactic molecules production (Keane and Strieter 2000). Although several cell types of the central nervous system (CNS) are able to secrete cytokines including microglia, astrocytes and neurons, there is evidence that peripherally-derived cells can contribute to brain inflammation and injury (Ghirnikar et al. 1998; Giulian et al. 1989). It has been shown that cytokines cross the blood-brain barrier (BBB), probably either by active transport or through leaky regions of endothelia, when BBB is

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compromised by a pathological condition. Thus, the CNS can be affected not only by inflammatory mediators produced in the brain, but also by the actions of mediators from the periphery (Pollmacher et al. 2002).

It has been shown that exposure of cultured endothelial cells (ECs) to Hcy leads to endothelial activation, resulting in the increased expression of chemokines (Poddar et al. 2001) and adhesion molecules (Wang et al. 2002). On the other hand, Upchurch et al. (1997) suggest that endothelial injury, caused by Hcy, may be due to oxidative stress, nitric oxide (NO) and disturbances in the anti-thrombotic activities of the endothelium. In this context, we have previously reported that an acute experimental model of hyperhomocysteinemia in rats, similar to that found in homocystinuria, induces oxidative stress, reducing antioxidant defenses and increasing lipid peroxidation (Wyse et al. 2002; Matté et al. 2004, 2009).

In order to verify whether high Hcy levels could alter inflammatory markers, in the present study we evaluated the effect of acute Hcy administration on inflammatory parameters such as cytokines (TNF- α , IL-1 β and IL-6), chemokine CCL2 (MCP-1), nitrite and acute-phase proteins [(C-reactive protein (CRP) and α_1 -Acid glycoprotein)] levels in hippocampus, cerebral cortex and serum. Differential leukocytes counts in the blood of rats were also performed.

Materials and methods

Animals and reagents

Sixty-five Wistar rats were obtained from the Central Animal House of the Departamento de Bioquímica, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil. Animals were maintained on a 12/12 h light/dark cycle in an air-conditioned constant room temperature ($22\pm 1^\circ\text{C}$) colony room. Rats had free access to a 20% (w/w) protein commercial chow and water. The NIH “Guide for the Care and Use of Laboratory Animals” (NIH Publication No. 80–23, revised 1996), and the official governmental guidelines in compliance with the Federação das Sociedades Brasileiras de Biologia Experimental were followed in all experiments. All chemicals were obtained from Sigma Chemical Co., St. Louis, MO, USA.

Acute homocysteine treatment

Wistar rats, aged 29 days, received a single subcutaneous injection of saline solution (control) or Hcy (0.6 $\mu\text{mol/g}$ body weight). *D,L*-Hcy was dissolved in 0.9% NaCl solution (saline) and buffered to pH7.4. Plasma Hcy concentration in rats subjected to this treatment achieved

levels similar to those found in homocystinuric patients (Streck et al. 2002; Matté et al. 2009; Mudd et al. 2001). Rats were sacrificed by decapitation without anesthesia 15 min, 1 h, 6 h or 12 h after the injection; serum was separated and brain was quickly removed and hippocampus and cerebral cortex were dissected.

Tissue preparation

For acquisition of serum, whole blood was centrifuged at $1,000\times g$ for 5 min and the serum was immediately removed. Hippocampus and cerebral cortex were homogenized 1:5 (w/v) in saline solution (0.9% NaCl). The homogenate was centrifuged at $800\times g$ for 10 min at 4°C and the supernatant was used in assays.

Cytokines (TNF- α , IL-1 β , IL-6) and chemokine CCL2 (MCP-1) assay

TNF- α , IL-1 β , IL-6 and MCP-1 levels in hippocampus, cerebral cortex and serum were quantified by a rat high-sensitivity enzyme-linked immunoabsorbent assays (ELISA) with commercially-available kits (Biosource®, Camarillo, CA).

Nitrite assay

Nitrite levels were measured using the Griess reaction; 100 μL of supernatant of hippocampus and cerebral cortex were mixed with 100 μL Griess reagent (1:1 mixture of 1% sulfanilamide in 5% phosphoric acid and 0.1% naphthylethylenediamine dihydrochloride in water) and incubated in 96-well plates for 10 min at room temperature. The absorbance was measured on a microplate reader at a wavelength of 543 nm. Nitrite concentration was calculated using sodium nitrite standards (Green et al. 1982).

Acute-phase protein assay

Acute-phase proteins (CRP and α_1 -Acid glycoprotein) in serum were determined by a colorimetric assay with commercially available kits (BioSystems® and Bioclin®, Brazil).

Differential leukocyte count

Cytological slide smears stained with May-Grunwald/Giemsa were used for leukocyte differential counts with a light microscope, and total leukocytes were diluted in Thoma solution (1:20) and counted in a Neubauer chamber by light microscopy (Frode-Saleh et al. 1999).

Protein determination

Protein concentration was measured by the method of Lowry et al. (1951) using bovine serum albumin as standard.

Statistical determination

Data were analyzed by the Student's *t* test for unpaired samples. All analyses were performed using the Statistical Package for the Social Sciences (SPSS) software. Differences were considered statistically significant if $p < 0.05$.

Results

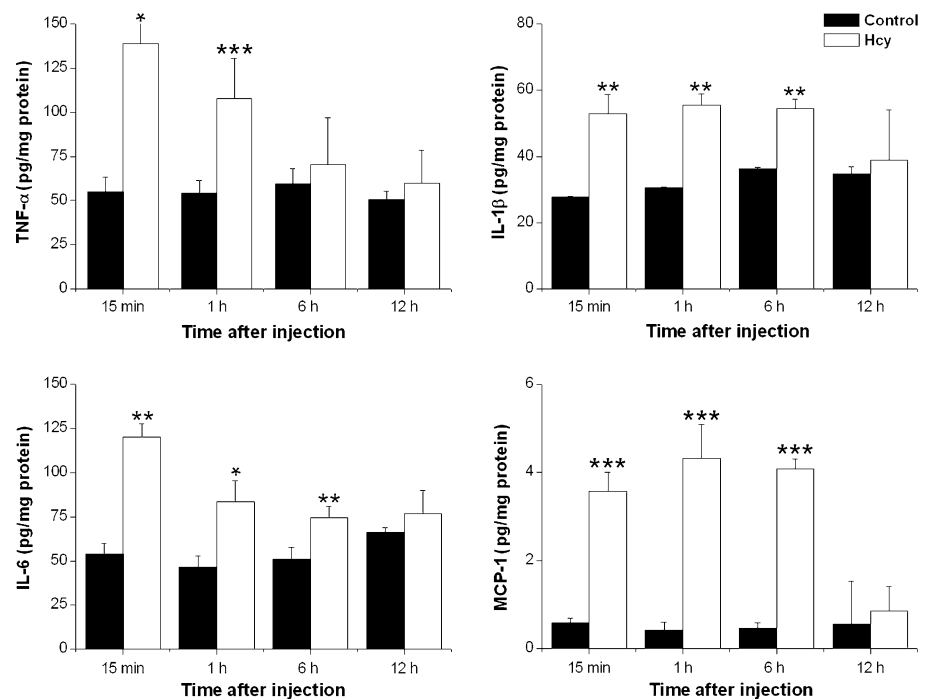
Firstly, we evaluated the effect of acute Hcy administration on cytokine levels (TNF- α , IL-1 β and IL-6) and chemokine CCL2 (MCP-1) in the hippocampus of rats. Figure 1 shows that Hcy significantly increased the levels of TNF- α (15 min: [$t(6)=8.17$; $p < 0.05$]; 1 h: [$t(6)=3.92$; $p < 0.001$], but not at 6 h: [$t(6)=0.67$; $p > 0.05$] after acute administration. IL-1 β and IL-6 levels also increased 15 min, 1 h and 6 h after Hcy injection, as compared to control (15 min: [$t(6)=5.72$; $p < 0.01$]; 1 h: [$t(6)=10.11$; $p < 0.01$]; 6 h: [$t(6)=8.49$; $p < 0.01$]); and (15 min: [$t(6)=10.30$; $p < 0.01$], 1 h: [$t(6)=3.90$; $p < 0.05$]; 6 h: [$t(6)=4.71$; $p < 0.01$]), respectively. We also observed that MCP-1 levels significantly increased (15 min: [$t(6)=11.17$; $p < 0.001$]; 1 h: [$t(6)=8.31$; $p < 0.001$] and 6 h: [$t(6)=24.17$; $p < 0.001$]) in the

hippocampus of rats after Hcy administration. Twelve hours after acute Hcy administration, no alterations in the levels of cytokines and chemokine CCL2 were observed (TNF- α [$t(6)=0.83$; $p > 0.05$]; IL-1 β [$t(6)=0.36$; $p > 0.05$]; IL-6 [$t(6)=1.08$; $p > 0.05$] and MCP-1 [$t(6)=0.94$; $p > 0.05$]).

We also investigated the effect of acute administration of Hcy on cytokines (TNF- α , IL-1 β and IL-6) and chemokine CCL2 (MCP-1) levels in the cerebral cortex of rats. Figure 2 shows that Hcy significantly increased TNF- α (15 min: [$t(6)=4.09$; $p < 0.05$]; 1 h: [$t(6)=8.88$; $p < 0.001$]; IL-1 β (15 min: [$t(6)=6.35$; $p < 0.01$]; 1 h: [$t(6)=5.24$; $p < 0.05$] and 6 h: [$t(6)=4.49$; $p < 0.05$]), and IL-6 levels (15 min: [$t(6)=5.52$; $p < 0.01$]; 1 h: [$t(6)=3.69$; $p < 0.05$] and 6 h: [$t(6)=3.24$; $p < 0.05$]) after acute administration, when compared to control. We also observed that Hcy administration significantly increased MCP-1 levels (15 min: [$t(6)=7.84$; $p < 0.001$]; 1 h: [$t(6)=11.52$; $p < 0.001$]). However, at 6 h after Hcy administration, we did not observe any alterations in TNF- α [$t(6)=1.47$; $p > 0.05$] and MCP-1 [$t(6)=2.24$; $p > 0.05$]. In addition, at 12 h after hyperhomocysteinemia the inflammatory parameters studied were not altered (TNF- α [$t(6)=0.06$; $p > 0.05$]; IL-1 β [$t(6)=1.08$; $p > 0.05$]; IL-6 [$t(6)=0.71$; $p > 0.05$] and MCP-1 [$t(6)=0.05$; $p > 0.05$]).

Next, we measured the nitrite levels after acute Hcy administration, in hippocampus and cerebral cortex of rats. Figure 3 shows that Hcy significantly increased the nitrite levels in hippocampus and cerebral cortex at 15 min, 1 h and 6 h after Hcy injection, as compared to control (15 min: [$t(6)=5.18$; $p < 0.01$]; 1 h: [$t(6)=4.14$; $p < 0.05$]; 6 h: [$t(6)=3.65$; $p < 0.01$]) and (15 min: [$t(6)=8.43$; $p < 0.001$]; 1 h:

Fig. 1 Effect of acute administration of homocysteine on cytokines (TNF- α , IL-1 β , IL-6) and chemokine CCL2 (MCP-1) levels in the hippocampus of rats. Results are expressed as mean \pm SD for six animals in each group. Different from control, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ (Student's *t*-test). Hcy: homocysteine; TNF- α : tumor necrosis factor alpha; IL-1 β : interleukin-1 beta; IL-6: interleukin-6; MCP-1: monocyte chemoattractant protein-1



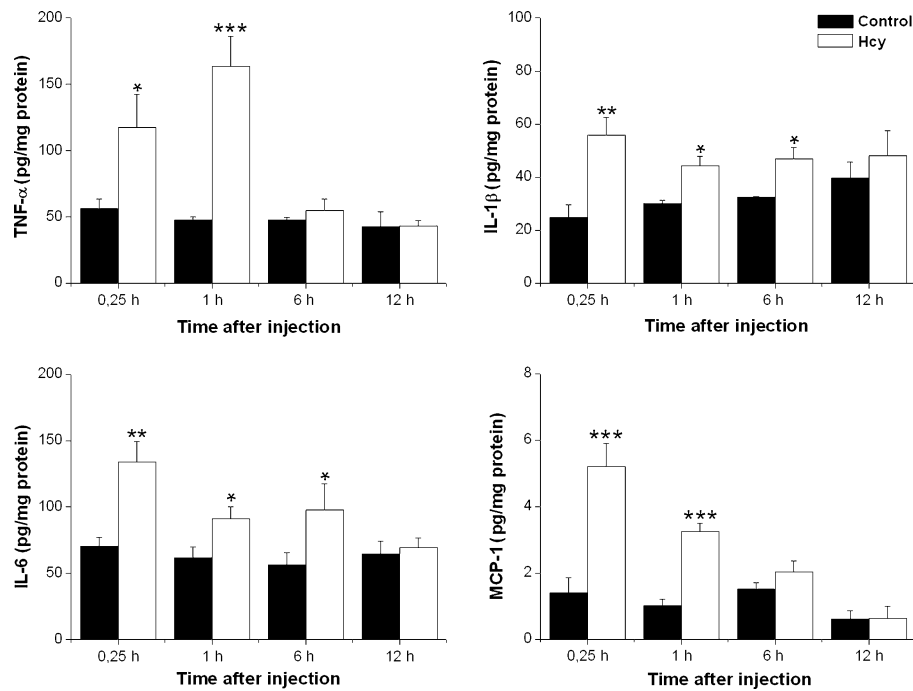


Fig. 2 Effect of acute administration of homocysteine on cytokines (TNF- α , IL-1 β , IL-6) and chemokine CCL2 (MCP-1) levels in the cerebral cortex of rats. Results are expressed as mean \pm SD for six animals in each group. Different from control, * p <0.05; ** p <0.01;

*** p <0.001 (Student's t -test). Hcy: homocysteine; TNF- α : tumor necrosis factor alpha; IL-1 β : interleukin-1 beta; IL-6: interleukin-6; MCP-1: monocyte chemoattractant protein-1

[$t(6)$ =3.35; p <0.05]; 6 h: [$t(6)$ =3.82; p <0.05]), respectively. Nitrite levels were not altered in the hippocampus [$t(6)$ =1.28; p >0.05] and cerebral cortex [$t(6)$ =0.32; p >0.05] of rats at 12 h after acute hyperhomocysteinemia.

We also evaluated the effect of acute administration of Hcy on cytokine (TNF- α , IL-6) levels in the serum of rats. Figure 4 shows that rats sacrificed at 15 min and 1 h after Hcy injection presented a significant increase in TNF- α levels in the serum, when compared to the control group, [$t(6)$ =2.76; p <0.05] and [$t(6)$ =3.82; p <0.05], respectively. However, animals sacrificed at 6 h: [$t(6)$ =1.26; p >0.05] and 12 h: [$t(6)$ =0.32; p >0.05] after Hcy administration did not present any alteration in this

parameter. In addition, IL-6 levels were increased at 15 min: [$t(6)$ =5.69; p <0.01], 1 h: [$t(6)$ =6.34; p <0.01] and 6 h: [$t(6)$ =10.13; p <0.001], but not at 12 h: [$t(6)$ =1.29; p >0.05] after Hcy administration.

The effects of acute administration of Hcy on acute-phase proteins (CRP and α_1 -Acid glycoprotein) in the serum of rats were also evaluated. Table 1 shows that serum CRP and α_1 -Acid glycoprotein levels were not altered at any time tested after acute Hcy administration (15 min: [$t(8)$ =0.14; p >0.05]; 1 h: [$t(8)$ =0.54; p >0.05]; 6 h: [$t(8)$ =1.41; p >0.05]; 12 h: [$t(8)$ =0.11; p >0.05]) and (15 min: [$t(8)$ =0.28; p >0.05]; 1 h: [$t(8)$ =1.82; p >0.05]; 6 h: [$t(8)$ =2.10; p >0.05]; 12 h: [$t(8)$ =0.04; p >0.05]), respectively.

Fig. 3 Effect of acute administration of homocysteine on nitrite levels in the hippocampus (a) and cerebral cortex (b) of rats. Results are expressed as mean \pm SD for six animals in each group. Different from control, * p <0.05; ** p <0.01; *** p <0.001 (Student's t -test). Hcy: homocysteine

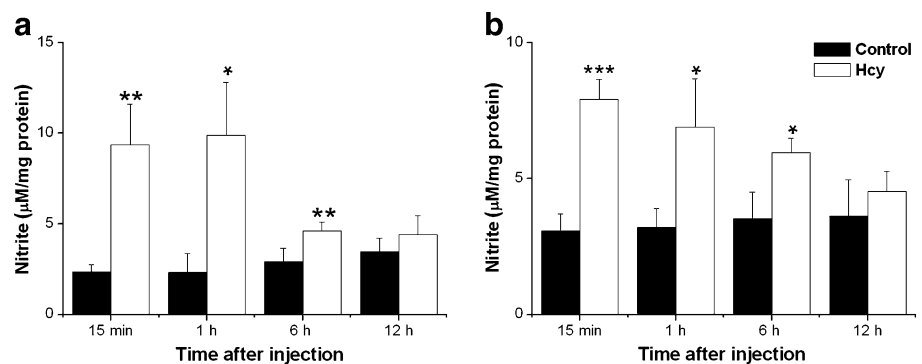
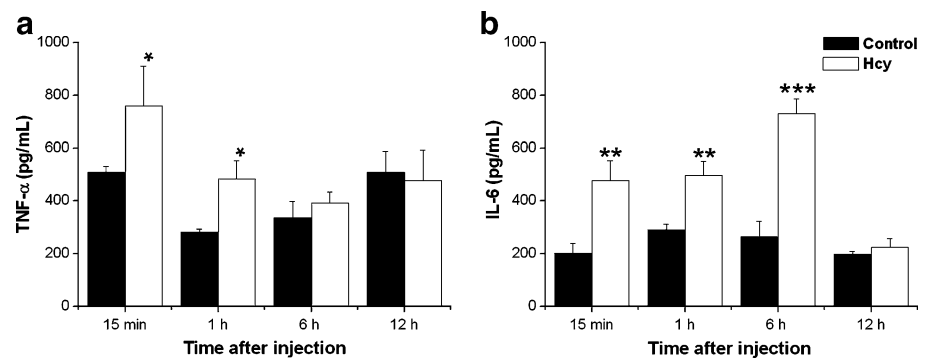


Fig. 4 Effect of acute administration of homocysteine on cytokine (TNF- α , IL-6) levels in the serum of rats. Results are expressed as mean \pm SD for six animals in each group. Different from control, * p <0.05; ** p <0.01; *** p <0.001 (Student's t -test). Hcy: homocysteine; TNF- α : tumor necrosis factor alpha; IL-6: interleukin-6



Finally, we investigated the effect of acute administration of Hcy on the differential count of blood leukocytes in rats. Table 2 shows a significant increase in the percentage of cells with a predominance of neutrophils in the blood (15 min: [$t(8)=2.89$; $p<0.01$]; 1 h: [$t(8)=2.27$; $p<0.05$]) and monocytes (15 min: [$t(8)=2.39$; $p<0.05$]; 1 h: [$t(8)=2.48$; $p<0.05$]) after Hcy administration, when compared to the control group. In addition, the percentage of neutrophils and monocytes was not altered after acute hyperhomocysteinemia (6 h: [$t(8)=0.22$; $p>0.05$]; 12 h: [$t(8)=1.97$; $p>0.05$]) and (6 h: [$t(8)=0.49$; $p>0.05$] and 12 h: [$t(8)=0.82$; $p>0.05$]), respectively. Nevertheless, the total number of leukocytes did not change significantly after Hcy administration, as compared to the control group (data not shown).

Discussion

Homocystinuria is an inborn error of metabolism, characterized by a severe deficiency of cystathionine β -synthase activity. Affected patients present tissue accumulation of Hcy and a variable symptomatology, including mental retardation, epilepsy, seizures and atherosclerosis, whose pathophysiology is poorly understood (Mudd et al. 2001).

Table 1 Effect of acute administration of homocysteine on acute-phase protein (CRP, α_1 -Acid glycoprotein) levels in the serum of rats

Group	CRP (mg/L)	α_1 -Acid glycoprotein (mg/dL)
Control 15 min	0.14 \pm 0.10	12.2 \pm 3.11
Hcy 15 min	0.13 \pm 0.73	11.60 \pm 3.01
Control 1 h	0.25 \pm 0.14	17.80 \pm 5.49
Hcy 1 h	0.20 \pm 0.09	12.70 \pm 4.43
Control 6 h	0.11 \pm 0.05	14.20 \pm 1.30
Hcy 6 h	0.19 \pm 0.08	10.50 \pm 3.64
Control 12 h	0.17 \pm 0.04	13.50 \pm 2.88
Hcy 12 h	0.18 \pm 0.10	13.42 \pm 2.50

Results are expressed as mean \pm SD for eight animals in each group (Student's t -test)

Hcy homocysteine, CRP C-reactive protein

Since Hcy and inflammatory parameters seem to be associated with the pathogenesis of several diseases (Gori et al. 2005; Weiss et al. 2003; Welch and Loscalzo 1998), in the present study, we evaluated the effects of acute hyperhomocysteinemia on important markers of inflammation such as cytokines, chemokine CCL2, nitrite levels and acute-phase proteins in hippocampus, cerebral cortex and serum of rats. In addition, in order to evaluate the blood leukocyte profile, differential counts of leukocytes were also performed. Firstly, we investigated the effect of Hcy administration on the hippocampus and cerebral cortex of rats. Results showed that Hcy increases the cytokines IL-1 β and IL-6, chemokine CCL2 (MCP-1) and nitrite levels in both cerebral structures studied from rats sacrificed at 15 min, 1 h and 6 h, whereas TNF- α was increased only at 15 min and 1 h after acute administration. However, 12 h after acute administration of Hcy, onwards, there was no effect on cytokines and chemokine CCL2. It is possible that the increase in the levels of TNF- α , IL-1 β , IL-6, MCP-1 and nitrite probably depend on Hcy, since a previous study showed that this amino acid presents a peak in the brain at 15 min after injection, returning to baseline levels after 12 h (Streck et al. 2002)

It has been reported that proinflammatory cytokines and other mediators play an essential role in CNS inflammation

Table 2 Effect of acute administration of homocysteine on the differential count of leukocytes (% of leukocytes) in blood of rats

Group	Neutrophils %	Lymphocytes %	Monocytes %
Control 15 min	17.50 \pm 4.23	80.33 \pm 4.08	2.00 \pm 0.63
Hcy 15 min	30.50 \pm 10.15**	68.00 \pm 9.87*	3.35 \pm 1.20*
Control 1 h	22.17 \pm 2.98	75.50 \pm 3.14	2.33 \pm 0.51
Hcy 1 h	29.00 \pm 6.73*	66.50 \pm 8.50*	4.50 \pm 2.07*
Control 6 h	22.50 \pm 5.84	74.33 \pm 6.37	3.17 \pm 0.75
Hcy 6 h	23.50 \pm 9.26	73.67 \pm 10.15	2.83 \pm 1.47
Control 12 h	21.80 \pm 2.86	74.80 \pm 3.03	3.20 \pm 1.09
Hcy 12 h	26.20 \pm 4.08	69.80 \pm 5.71	4.00 \pm 1.87

Results are expressed as mean \pm SD for eight animals in each group (Student's t -test)

Hcy homocysteine

(Rothwell and Luheshi 2000). Activated microglia may secrete a diverse range of proinflammatory cytokines and neurotoxic factors, such as NO and reactive species of oxygen (ROS), which contribute to neuronal damage in neurodegenerative diseases (Liu and Hong 2003; Lerouet et al. 2002). In this context, hyperhomocysteinemia has been demonstrated to induce neuronal death, often associated with increased levels of ROS formation (Lipton et al. 1997). Indeed, we have previously demonstrated that chronic Hcy treatment induces oxidative stress in the cerebrum of rats, increasing lipid peroxidation and reducing enzymatic and non-enzymatic antioxidant defenses (Matté et al. 2007, 2009; Streck et al. 2003). Therefore, these findings may be closely related to the increase in proinflammatory cytokines and nitrite levels elicited by Hcy in brain, observed in the present study, since proinflammatory cytokines are often produced in response to oxidative stress and, conversely, may act to cause oxidative stress in their target cells (Halliwell and Gutteridge 2007).

Other reports are in agreement with our data showing a relationship between hyperhomocysteinemia and proinflammatory state (Gori et al. 2005; de Jong et al. 1997). Some authors have shown that Hcy treatment stimulates MCP-1 expression in several cell types (Sung et al. 2001, Wang et al. 2000, 2001). In addition, the pre-treatment of cells with nuclear factor Kappa β (NF- κ B) inhibitors can alleviate the stimulatory effect of Hcy on MCP-1 expression, supporting the notion that Hcy-stimulated chemokine expression is mediated via NF- κ B activation (Wang et al. 2000, 2001; Au-Yeung et al. 2003). In turn, NF- κ B plays a key role in the orchestration of inflammatory and immune responses by controlling transcription of genes encoding adhesion molecules and cytokines (Yun et al. 2009; Zhou et al. 2007; Dalal et al. 2003).

A number of studies have indicated that Hcy may contribute to the progression of atherosclerosis, in part by enhancing vascular inflammation (Sung et al. 2001; Wang et al. 2000, 2001). Hcy has been shown to promote a potent stimulation of IL-6 expression in cultured rat aorta vascular smooth muscle cells (VSMCs) (Zhang et al. 2006). In vitro studies have shown that Hcy is able to induce mRNA and protein expression of the proinflammatory cytokines, IL-8 and MCP-1 in cultured human aortic endothelial cells (HAECs) (Poddar et al. 2001; Sung et al. 2001). In contrast, it had no effect on the expression of other cytokines, such as TNF- α , IL-1 β , granulocyte/macrophage colony-stimulating factor (GM-CSF) and transforming growth factor- β (TGF- β) (Poddar et al. 2001).

In order to verify the systemic effects of Hcy on inflammation, we also measured the levels of TNF- α and IL-6 in the serum of rats submitted to acute hyperhomocysteinemia. Results revealed that Hcy significantly increases these cytokines, where IL-6 levels increased at

15 min, 1 h and 6 h, whereas TNF- α was increased only at 15 min and 1 h after administration. Similarly to effects observed in the brain, we did not observe any alteration at 12 h after Hcy administration.

It has been shown that Hcy may promote endothelial dysfunction, probably associated with oxidative stress, which can lead to the activation of proinflammatory pathways in the vasculature (Weiss 2005; Zhang et al. 2001; Liu et al. 2008). TNF- α is one of the central mediators of tissue inflammation and induces synthesis of other inflammatory cytokines (Lucas et al. 2006), whilst elevated IL-6 levels are closely related to an increased risk of myocardial infarction (Lindmark et al. 2001; Ridker et al. 2000a); as such, our findings that demonstrate that Hcy increases both TNF- α and IL-6 levels in serum of rats suggest an important role of Hcy in the induction of a systemic inflammatory response.

On the other hand, the local release of TNF- α can lead to activate neutrophils and endothelial cells to further upregulate the expression of adhesion molecules so that activated neutrophils can bind and migrate across the endothelial cell barrier (Argenbright and Barton 1992). Moreover, it has been demonstrated that Hcy in human can enhance the adhesion of leukocytes to the vascular endothelium and lead to leukocyte-mediated changes in endothelial integrity and function, ultimately resulting in thromboses and vascular lesion (Dudman et al. 1999). Together, these events facilitate the initiation and progression of atherosclerosis lesion (Weiss 2005; Napoli et al. 2001) and might explain why mild hyperhomocysteinemia has been described as an important risk factor for neurodegenerative and vascular diseases (Mattson and Shea 2003).

Acute-phase proteins are produced by the liver in large quantities during an inflammatory state (Patti et al. 2002). Furthermore, it has been suggested that acute-phase proteins have an essential role in the inhibition of extracellular proteases, blood clotting, fibrinolysis and modulation of immune cell function (Ridker et al. 2000b). Considering that we have previously reported that histological analysis reveals the presence of inflammatory infiltrate in liver tissue sections from hyperhomocysteinemic rats (Matté et al. 2009); in this study, acute-phase proteins, CRP and α 1-Acid glycoprotein, were also evaluated. However, results showed that Hcy did not alter these parameters at any time tested. These data are in agreement with clinical findings from other investigators who did not observe alterations in the CRP levels in older subjects with hyperhomocysteinemia (Gori et al. 2005).

Regarding the blood leucocyte profile, the analysis of differential counts of leukocytes showed an increase in the relative number of neutrophils and monocytes at 15 min and 1 h, but not at 6 h or 12 h, after Hcy administration. However, the total number of leukocytes did not change

(data not shown). It has been reported that cytokines can induce neutrophilic inflammation (Jatakanon et al. 1999); on the other hand, neutrophils can also be important sources of cytokines such as TNF- α (Thomas et al. 1995). Interestingly, we found that Hcy increased TNF- α levels at the same time point that neutrophilia was observed (15 min and 1 h after Hcy injection), suggesting a possible relationship between TNF- α and the increase in neutrophils. However, further studies are needed to determine the mechanism of neutrophilia and monocytosis elicited by Hcy.

In summary, in the present study, we demonstrated that acute Hcy administration induces immune activation by increasing cytokines, chemokine and nitrite levels in the hippocampus, cerebral cortex and serum of rats, in addition to increasing the relative number of neutrophils and monocytes in the blood. Our findings provide insights into the role of Hcy in the pathogenesis of human vascular inflammation, cerebrovascular and neurodegenerative disease and facilitate the identification of new therapeutic approaches in the treatment of homocystinuric patients. However, many questions and cellular mechanisms by which hyperhomocysteinemia exert these effects remain to be answered.

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