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Influence of Homocysteine on Metabolic Processes in Biological Systems

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Abstract

The study is devoted to determining the level of homocysteine in mammals and determining the extent of its influence on metabolic processes.

Homocysteine is a sulfur-containing non-proteinogenic amino acid that is formed as a result of the oxidation-reduction metabolism of methionine. Homocysteine metabolism includes transmethylation, remethylation, and transsulfation reactions. When metabolism is disrupted, homocysteine formation increases, which leads to hyperhomocysteinemia.

In this work, homocysteine levels were determined in rats with experimental heart failure and experimental chronic kidney disease in rats. Chronic heart failure was modeled by intraperitoneal administration of phenylephrine for 28 days. Chronic kidney disease was modeled by 5/6 nephrectomy. The residual kidney resection model was performed in two sessions under 2 % isoflurane anesthesia: during the first week, approximately 2/3 of the left kidney was removed from the rats, and during the second week, the entire right kidney was removed. The level of homocysteine, creatinine, urea, potassium, sodium, chlorine ions, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase activity were determined in the blood 2 and 6 months after pathology modeling.

Based on the results of the studies, it was established that an increase in homocysteine levels is observed in a number of pathologies, such as chronic heart failure and chronic kidney disease. An increase in the concentration of homocysteine in the blood plasma was revealed as the pathology progressed. The level of homocysteine in plasma correlates with the level of creatinine and urea and is absolutely not associated with the level of sodium, potassium, chloride ions and the activity of metabolic enzymes. The results of the study allow us to recommend measuring the concentration of homocysteine in plasma to determine the degree of metabolic disorders.

Keywords: homocysteine, blood, metabolites, creatinine, urea, heart, kidneys, biological systems, enzymes, ions.

1. Introduction

Homocysteine (Hcy) is a sulfur-containing non-proteinogenic amino acid that is formed as a result of the redox metabolism of methionine. Hcy metabolism involves transmethylation,

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remethylation, and transsulfation reactions. In most cells, through the transmethylation of Hcy in the methionine metabolic cycle, the methyl group of activated methionine (S-adenosylmethionine or SAM) is attached to methyl acceptors (DNA, RNA, and protein) by methyltransferases, and S-adenosyl-Hcy (SAH) is rapidly hydrolyzed to adenosine and Hcy, resulting in hyperhomocysteinemia. Once formed, Hcy can be recycled to methionine or converted to cysteine by remethylation and transsulfation, respectively. Hcy is remethylated to methionine through two separate reactions catalyzed by three different enzymes. In all tissues, folate donates a methyl group via methylenetetrahydrofolate reductase (MTHFR) in a reaction catalyzed by methionine synthase, a vitamin B12-dependent enzyme (Esse et al., 2019). Otherwise, mainly in the mammalian heart, liver, and kidney, Hcy is remethylated using betaine, which donates a methyl group by betaine-Hcy S-methyltransferase (BHMT). Betaine is found in some foods such as wheat germ or bran, spinach, beets, seafood, and legumes. Studies have confirmed the ability of betaine to reduce Hcy levels in the presence of excess methionine intake, and the fact that low-dose betaine supplementation results in immediate and long-term reductions in plasma Hcy levels in healthy individuals (Steenge et al., 2003, Olthof et al., 2003). The remethylation process begins at low concentrations of Hcy and methionine (McRae, 2013). On the other hand, mainly in the liver, but also in the kidneys, small intestine and pancreas (Zaric et al., 2019), Hcy is enzymatically modified by cystathionine β-synthase, a vitamin B6-dependent enzyme, to irreversibly form cysteine via the intermediate cystathionine. The transsulfuration pathway results in the formation of sulfur metabolites including GSH, a key cellular antioxidant, and hydrogen sulfide (H_2S), which acts as a gaseous signaling molecule. The transsulfuration pathway becomes active when Hcy and methionine concentrations increase (Verhoef et al., 2005).

In plants, Hcy is synthesized by two pathways. One involves the plastid/chloroplast and involves a pathway from sulfate via the formation of cysteine and cystathionine (CysT); however, next to cysteine, also O-phosphohomoserine can be metabolized to CysT by CysT γ-synthase. β-cleavage of CysT to Hcy is catalyzed by cystathionine β-lyase (CBL) (Ravanel et al., 2005). The other cytosolic pathway involves the formation of Hcy as a by-product of the methylation reaction in plant cells (Jakubowski, 2006). In this regard, S-adenosylhomocysteine (AdoHcy) is converted to Hcy in a reaction catalyzed by S-adenosylhomocysteine hydrolase (SAHH) (Ravanel et al., 2005).

Most studies confirm an increase in homocysteine levels in various pathologies, but data on the correlation of homocysteine levels with metabolic parameters remain unstudied.

The aim of our research was to study the correlation of homocysteine levels with metabolic parameters of the blood in experimental chronic failure and chronic renal disease in rats.

2. Methodology

Modeling of chronic heart failure (CHF) and chronic kidney disease was performed on male Wistar rats. All experimental protocols complied with international ethical standards for the humane treatment of animals and were approved by the Local Ethics Committee of KFU (protocol 33 dated 11/25/2021). The animals were housed according to the guidelines for animal research, with constant room temperature, a 12-hour light/dark cycle, and 50±5 % humidity, as well as standard chow and water ad libitum.

The animals were divided into 3 groups:

1. Control (n = 16)
2. Rats with experimental chronic heart failure (n = 16)
3. Rats with experimental chronic kidney disease (n = 16).

Chronic heart failure was modeled by intraperitoneal administration of phenylephrine for 28 days (Rajanathan et al., 2022).

Chronic kidney disease was modeled by 5/6 nephrectomy. The residual kidney resection model was performed in two sessions under 2 % isoflurane anesthesia: in the first week, approximately 2/3 of the left kidney was removed from the rats, and in the second week, the entire right kidney was removed. After surgery, the rats were given free access to tap water and standard rat chow (Nishiyama et al., 2019).

The level of homocysteine, creatinine, urea, potassium, sodium, chlorine ions, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase activity were measured in the blood 2 and 6 months after pathology modeling. The level of homocysteine was determined in blood plasma by a colorimetric method on a Thermo Scientific Multiskan F analyzer using a kit

from Elabscience Biotechnology Co., Ltd. Biochemical analysis of blood plasma was performed by photometric method using commercial kits.

To test the normal distribution of data, Fisher's F-test and Shapiro-Wilk test were used using OriginPro 8.5 software. To compare two independent groups and paired data, Mann-Whitney U-test and Wilcoxon paired test were used, respectively. Correlation analysis was performed using Pearson coefficient.

3. Results and discussion

After modeling heart failure, rats developed cardiomegaly (Figure 1).

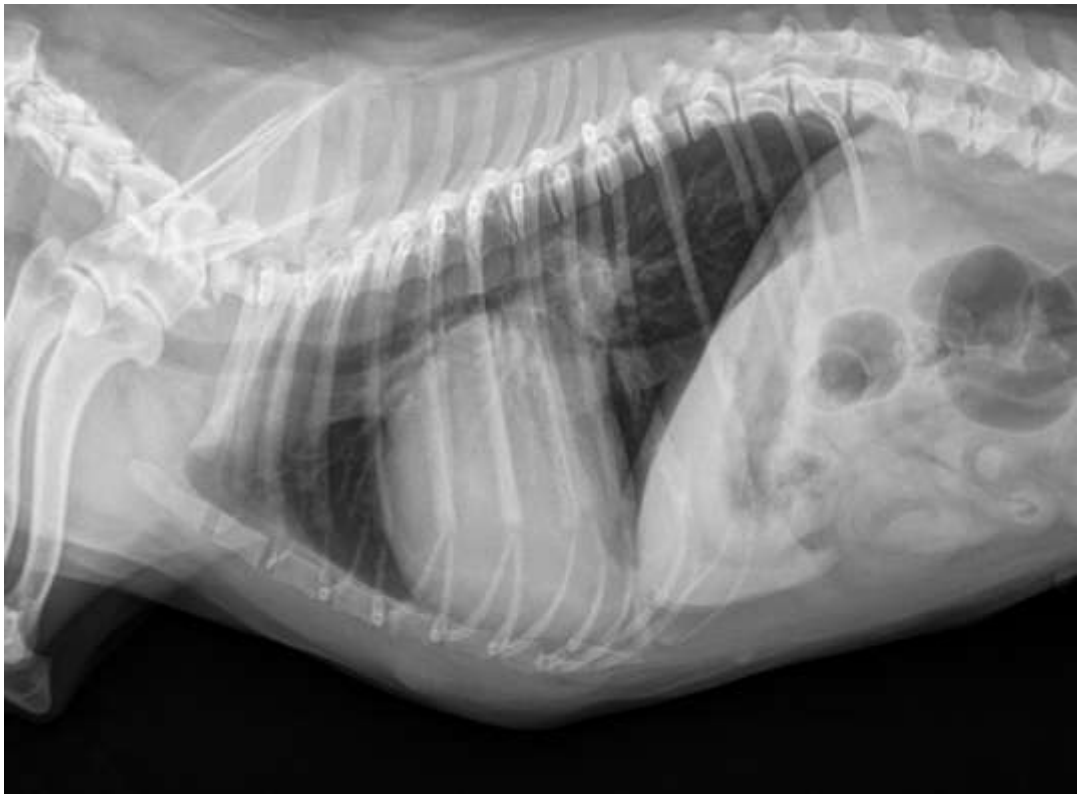

Fig. 1. Lateral radiograph of a rat with left-sided chronic heart failure. Severe cardiomegaly, especially in the left atrium

The studies showed that the level of homocysteine in the blood plasma gradually increased with the development of chronic heart failure.

In healthy rats of the control group, the level of homocysteine in the blood was 6.75 ± 0.25 μmol/l. Immediately after the end of the modeling, the level of homocysteine did not change, while clinical symptoms of heart failure were pronounced.

Two months after the modeling of heart failure, the level of homocysteine increased to 14.9 ± 0.66 μmol/l, and after 6 months to 21.5 ± 0.7 μmol/l (Figure 2).

This fact calls into question the claims of many authors that hyperhomocysteinemia is a risk factor for cardiovascular diseases (Jakubowski, 2019; Kim et al., 2018; Kubota et al., 2019). Our data confirm the studies of Wang X et al. (2023), who found no causal relationship between elevated plasma homocysteine levels and cardiovascular diseases.

At the same time, the study of metabolic parameters showed that the level of homocysteine correlated with the level of creatinine in the blood, which also increased as chronic heart failure developed. The remaining parameters studied were independent of the level of plasma homocysteine (Table 1).

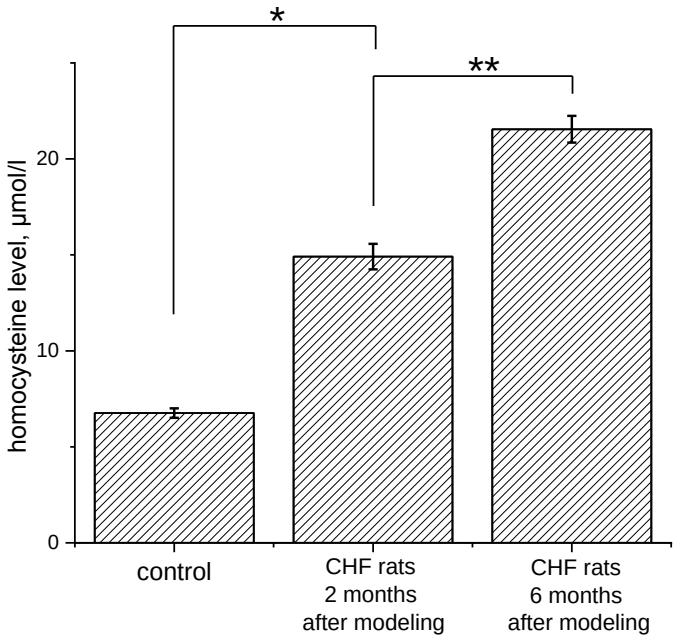

Fig. 2. Homocysteine levels in rats with experimental chronic heart failure,
*– is the significance of differences p≤0.05

Table 1. Changes in the biochemical composition of blood in rats with CHF

Parameter	Control	CHF rats 2 months after modeling	CHF rats 6 months after modeling
Urea, mmol/l	6.8±0.7	8.4±0.71*	8.3±0.71
Creatinine, µmol/l	71.6±8.2	136.1±18.4*	158.6±19.0*
AST, U/l	20.6±2.07	40.2±3.5	77.5±6.5*
ALT, U/l	40.6±2.62	38.6±2.9	40.2±2.9
LDH, U/l	21.7±1.19	29.8±3.9	31.7±6.9
Na, mmol/l	156.3±3.06	148.5±0.92	152.4±0.92
K, mmol/l	4.15±0.94	3.69±0.48	3.89±0.48
Cl, mmol/l	90.5±4.5	86.2±5.28	99.2±1.28

*– is the significance of differences p≤0.05

Experimental chronic kidney disease was also accompanied by an increase in the level of homocysteine in the blood. In experimental chronic kidney disease, it is higher than in chronic heart failure. Two months after modeling, the concentration of homocysteine in the blood plasma is 17.46±0.57 µmol/l, and after 4 months it increases to 30.25±0.68 µmol/l (Figure 3). A positive correlation with the level of creatinine and urea in the blood was revealed (Table 2).

Therefore, the level of homocysteine increases with the progression of renal pathology and can be used as a marker for predicting the development of the severity of renal failure.

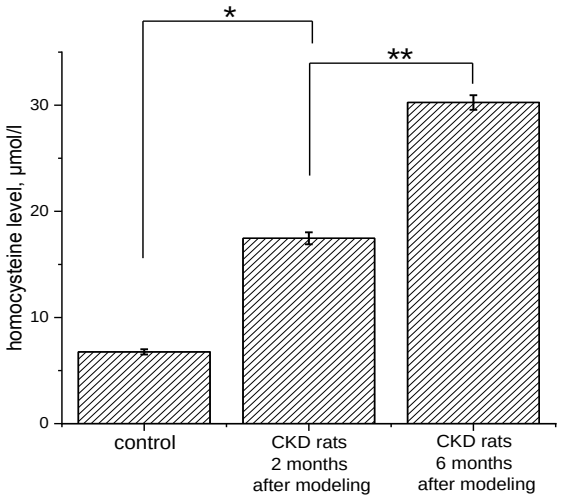

Fig. 3. Homocysteine levels in rats with experimental chronic kidney disease,
* – is the significance of differences p≤0.05

Table 2. Changes in the biochemical composition of blood in rats with CKD

Parameter	Control	CHF rats 2 months after modeling	CHF rats 6 months after modeling
Urea, mmol/l	6.8±0.7	36.8±2.7*	53±2.7*
Creatinine, µmol/l	71.6±8.2	186±9.4*	221±9.4*
AST, U/l	20.6±2.07	20.8±2.47	22±2.47
ALT, U/l	40.6±2.62	42.6±2.82	48±2.82
Na, mmol/l	156.3±3.06	162.5±3.06	170±3.06
K, mmol/l	4.15±0.94	3.65±0.94	3.15±0.94
Cl, mmol/l	90.5±4.5	85.4±4.5	79±4.5

*– is the significance of differences p≤0.05

There is sufficiently strong clinical evidence that hyperhomocysteinemia does not cause renal failure (Samuelsson et al., 1999; Sarnak et al., 2002; Hovind et al., 2001), although a recent study has linked higher homocysteine levels with a greater decrease in glomerular filtration rate (Ninomiya et al., 2004). Therefore, the association between hyperhomocysteinemia and renal dysfunction may be causal, i.e. renal failure causes elevated plasma homocysteine levels, but this association may also be due to other co-factors that, on the one hand, lead to renal dysfunction and, on the other hand, cause hyperhomocysteinemia through different mechanisms. Evidence for the association between hyperhomocysteinemia and the progression of chronic renal failure is the lack of a significant relationship between enzyme activity (aspartate aminotransferase, alanine aminotransferase) and the concentration of plasma ions in the blood (sodium, potassium, chloride ions).

4. Conclusion and recommendations

An increase in the homocysteine level in biological systems is an indicator of metabolic disorders. The concentration of homocysteine in the blood plasma of mammals positively correlates with the final metabolites of blood plasma, which allows it to be used to predict the development of a number of pathologies, including chronic heart failure and chronic renal disease.

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