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The relationship of folate deficiency, hyperhomocysteinemia and glutathione metabolism in hypertensive patients

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Abstract

Hypertension (HTN) is often accompanied by folic acid (FA) deficiency and hyperhomocysteinemia (HHcy). Reduced glutathione (GSH) and dependent enzymes determine the state of cellular antioxidant and redox systems in cardiovascular pathology. **The aim** of our work is to assess the relationship between the status of FA and the presence of HHcy with enzymes of glutathione metabolism and the redox state of erythrocyte glutathione in HTN. **Design and methods.** In blood plasma samples from 43 HTN patients admitted to the clinic of Pavlov University, the concentration of FA and total homocysteine (oHcy) was determined. We also evaluated the level of GSH, the activity of glutathione peroxidase and glutathione reductase (GR) in erythrocytes. **Results.** In the whole group, GR activity positively correlated with the concentration of FA (R = 0,415; p = 0,001). A significant decrease in GR activity (U/g Hb) was found in the subgroup with the low level of FA [0,8 (0,5–1,1)] compared with the subgroup without a FA deficiency [1,2 (0,9–2,0)]. The GSH level (μM/g Hb) was also lower (p < 0,018) in the subgroup with FA deficiency [1,3 (0,9–2,1)] compared with the subgroup with normal FA levels [1,8 (1,5–4,6)]. A significant decrease in the level of GSH and GR activity in the subgroup with HHcy was found compared with the corresponding parameters in the subgroup without HHcy. However, even in the absence of HHcy patients with FA deficiency demonstrated a significant decrease in GR activity compared to patients without FA deficiency. In this case, GR positively correlated with FA (R = 0,564; p = 0,03). **Conclusions.** The deficiency of FA can increase the deficiency of GR activity, regardless of the level of oHcy. The indicator of GR activity in erythrocytes can be considered as a possible marker of functional deficiency of FA in the absence of HHcy.

Key words: hypertension, folic acid, hyperhomocysteinemia, glutathione, glutathione reductase, red blood cells

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Взаимосвязь дефицита фолатов, гипергомоцистеинемии и метаболизма глутатиона у больных артериальной гипертензией

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Резюме

Актуальность. Артериальная гипертензия (АГ) нередко сопровождается дефицитом фолиевой кислоты (ФК) и гипергомоцистеинемией (ГГЦ). Глутатион восстановленный (ГлВ) и зависимые от него ферменты определяют состояние клеточной антиоксидантной и окислительно-восстановительной систем при сердечно-сосудистой патологии. **Цель работы** — оценить взаимосвязь статуса ФК и наличия ГГЦ с ферментами метаболизма глутатиона и окислительно-восстановительным состоянием глутатиона эритроцитов при АГ. **Материалы и методы.** В образцах крови от 43 больных АГ, находившихся на стационарном лечении в клиниках ПСПбГМУ им. И. П. Павлова, определяли концентрацию ФК, общего гомоцистеина (оГци) в плазме, а также содержание ГлВ, активность глутатионпероксидазы и глутатионредуктазы (ГР) в эритроцитах. **Результаты.** В основной группе активность ГР положительно коррелировала с концентрацией ФК ($R = 0{,}415$; $p = 0{,}001$). Выявлено статистически значимое снижение активности ГР (в Ед/г Hb) в подгруппе с пониженным уровнем ФК [0,8 (0,5–1,1)] по сравнению с подгруппой без дефицита ФК [1,2 (0,9–2,0)]. Уровень ГлВ (в мкМ/г Hb) был также ниже ($p < 0{,}018$) в подгруппе с недостаточностью ФК [1,3 (0,9–2,1)] по сравнению с подгруппой с нормальным уровнем ФК [1,8 (1,5–4,6)]. Установлено статистически значимое снижение уровня ГлВ и активности ГР в подгруппе с ГГЦ по сравнению с соответствующими параметрами в подгруппе без ГГЦ. Однако даже в отсутствие ГГЦ у больных с дефицитом ФК обнаружено статистически значимое понижение активности ГР по сравнению с больными без дефицита ФК. При этом ГР положительно коррелировала с ФК ($R = 0{,}564$; $p = 0{,}03$). **Заключение.** Дефицит ФК может усиливать недостаточность активности ГР независимо от уровня оГци. Показатель активности ГР в эритроцитах может рассматриваться как возможный маркер функционального дефицита ФК в отсутствии ГГЦ.

Ключевые слова: артериальная гипертензия, фолиевая кислота, гипергомоцистеинемия, глутатион, глутатионредуктаза, эритроциты

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Introduction

Numerous studies have shown that cardiovascular diseases (CVD), including arterial hypertension (HTN), are accompanied by a deficiency of folic acid (FA), which has great potential for preventing their development [1–3]. The most active FA derivative 5-methyltetrahydrofolate (5-MTHF), which is a product of an enzymatic reaction catalyzed by methylenetetrahydrofolate reductase (MTHFR), participates in the transfer of one-carbon groups and plays a key role in the homocysteine remethylation into methionine, thereby providing biochemical methyl groups [4]. Almost all enzymes of methylation reactions depend on the coenzyme S-adenosyl-methionine. The transfer of the methyl group from S-adenosyl-methionine to the deoxycytosine residue in DNA is involved in the regulation of gene expression, transcription, and repair, and, in general, in the mechanism of epigenetic phenotype formation [3]. Due to the deficiency of 5-MTHF, hyperhomocysteinemia (HHcy) occurs and, as you know, the level of total homocysteine (tHcy) in blood plasma is an independent risk factor for CVD, including HTN [5–7]. A large number of modern studies are devoted to the problem of optimal consumption of FA associated with a decrease in the risk of chronic diseases, including hypertension [8–11]. The norms of consumption of FA are estimated in relation to the concentration of tHcy, which is considered as an important indicator of functional deficiency of FA [2, 6]. In turn, to reduce the level of tHcy in HHcy, large doses of FA are used to reduce the risk of complications in CVD and during pregnancy. It is believed that the beneficial effect of FA on vascular functions is directly related to the mechanism of decreasing the level of tHcy [8]. However, recent studies have demonstrated the beneficial effects of FA not associated with a decrease in tHcy [12], which indicates the presence of alternative mechanisms. For example, folates can interact with the endothelial enzyme NO synthase and affect the bioavailability of the cofactor NO and, therefore, reduce the formation of peroxynitrite, the most aggressive prooxidant [13]. Numerous publications testify to the role of glutathione in the development of hypertension [14–17]. Reduced glutathione (GSH) is the most important intracellular regulatory peptide, the main antioxidant and a homeostasis factor for the redox potential of the cell. GSH-dependent enzymes are involved in the prevention and limitation of oxidative stress (OS) [18, 19]. Glutathione homeostasis in the cell is supported by the coordinated action of glutathione peroxidase (GPO) and glutathione reductase (GR). At the same time, GPO uses GSH as a cosubstrate to restore the excess of hydroperoxides and lipid peroxides. The main regulatory function of GR is to convert oxidized glutathione into its reduced form in order to maintain the redox potential of the cell and use it in enzymatic reactions. For the diagnosis of FA deficiency, the World Health Organization has identified biomarkers — the concentration of folate in erythrocytes, the concentration of folate and homocysteine in serum / plasma, which can be used for therapeutic drug monitoring when prescribing folic acid. Excessive consumption of FA and an increase in its concentration in the blood increases the risks of the epigenetic effects of this vitamin. The study of the causes of folate deficiency, the development of biomarkers of folate status is important for therapeutic monitoring when using FA for the prevention of CVD. In connection with the known pathogenetic role of Hcy, deficiency of FA and OS, the goal was to assess the relationship between the status of FA and the presence of HHcy with the enzymes of glutathione metabolism and the redox state of glutathione in erythrocytes in hypertension.

Materials and methods

We used blood samples from 43 patients with HTN (Table 1) who were hospitalized in the clinics of Pavlov University. The study did not include patients with megaloblastic anemia, decreased vitamin B 12 levels and laboratory signs of iron deficiency. The comparison group consisted of blood samples from 32 donors of similar age without hypertension, signs of inflammation and chronic diseases in history. In all cases, there was informed consent of the subjects to anonymous use of the data obtained, and the study protocol was approved by the ethics committee of Pavlov University. The study material was blood taken from the cubital vein with heparin as an anticoagulant.

The blood was centrifuged for 15 min at 580 g. Erythrocytes were washed twice with cold saline, frozen, and stored in a freezer at –82 °C until analysis. In 10 % hemolysates, the activity of the enzymes GPO, GR and the concentration of GSH were determined [20] and calculated per gram of hemoglobin. The hemoglobin concentration in 10 % hemolysate was measured by the hemoglobin cyanide method using reagent kits from Sintacon (Russia). The redox potential of erythrocytes was assessed by the level of GSH and GR activity. To determine the concentration of tHcy in plasma, we used the method of liquid chromatography, which we described earlier [7, 21]. The

Table 1

CLINICAL CHARACTERISTICS OF THE SUBJECTS INCLUDED IN THE STUDY

Parameters*	Hypertensive patients	Comparison group, reference range
Number of subjects	43	32
men / women	11/32	12/20
Age, year	61 (45–70)	55 (42–58)
SBP, mmHg	130 (125–150)	100–130
DBP, mmHg	80 (80–90)	< 80
Antihypertensive therapy	yes	no
Total cholesterol, mmol/L	4.9 (4.1–5.78)	3.5–5.5
Obesity 1 degree	11%	нет
Obesity 2 degree	4.6%	нет
Plasma glucose, mmol/L	5.2 (4.7–6.0)	3.9–6.1
DM/IGT	6/3	нет
Creatinine, μmol/L, men	0.104 (0.076–0.135)	0.053–0.106
Creatinine, μmol/L, women	0.073 (0.058–0.109)	0.044–0.097
Glomerular filtration rate, ml/min	89 (25–98)	> 90
Plasma Urea, mM	6.35 (4.4–11.8)	2.9–7.5
Total protein, g/L	69 (65–73)	65–85
ALAT, U/L	16 (11–20)	До 40
ASAT, U/L	18 (15–21)	До 42
Fibrinogen, g/l	3.2 (2.7–4.3)	1.8–3.5
Erythrocytes, x1012 / l	4.2 (3.6–4.7)	(4.1–5.1)
Hemoglobin, g/l	124.5 (113.2–134.8)	132–164
Color index	0.89 (0.81–0.94)	0.85–1.05
Vitamin B12, pmol/L	285 (177–409)	133–679
tHcy, μmol/L	12.6 (8.0–19.0)	8.1 (6.5–10.9)
FA, nmol/L	13.8 (10.6–18.3)	> 13.4

Note: * median IQR; SBP — systolic blood pressure; DBP — diastolic blood pressure; DM — diabetes mellitus; IGT — impaired glucose tolerance; ALAT — alanine aminotransferase; AST — aspartate aminotransferase; tHcy — total homocysteine; FA — folic acid.

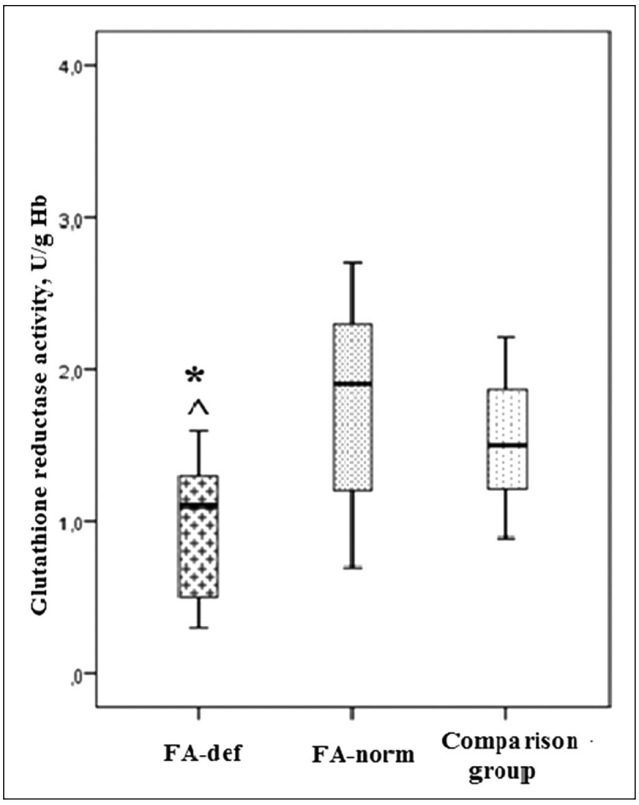
Table 2

GLUTATHIONE METABOLISM PARAMETERS IN PATIENTS WITH ARTERIAL HYPERTENSION WITH NORMAL (SUBGROUP 1) AND LOWERED (SUBGROUP 2) FOLIC ACID LEVELS

Parameters	Comparison group (N = 32)	Subgroup of Patients htn without hhcy (N = 19)	Subgroup of patients htn with hhcy (N = 24)
GSH, μM/g Hb.	3.3 (2.4–3.8)	1.8 (1.5–4.6) p* = 0.005	1.3 (0.9–2.1) p* = 0.001 p** = 0.018
GR, U/g Hb	1.57 (1.23–2.06)	1.2 (0.9–2.0) p* = 0.001	0.8 (0.5–1.1) p* = 0.001 p = 0.002**
GPO, U/g Hb	15.05 (10.9–18.33)	6.2 (4.8–9.1) p* = 0.001	7.3 (6.3–9.3) p* = 0.001
tHcy, μmol/L	8.1 (6.5–10.9)	9.75 (7.03–13.95)	13.9 (9.55–22.85)
FA (nmol/L)	> 13.4	22.45 (16.95–34.75)	11.7 (9.75–13.55)
Vitamin B12 (pmol/L)	133–679	293 (186–410)	138 (198–416)

Note: GSH — reduced glutathione; GR — glutathione reductase; GPO — glutathione peroxidase; tHcy — total homocysteine; FA — folic acid; P * — the level of statistical significance of differences when comparing the parameters of the subgroup with the comparison group; P ** — the level of statistical significance of differences when comparing the parameters of subgroups with each other.

Figure 1. Effect of folate deficiency in the activity of glutathione reductase in patients with arterial hypertension in the absence of hyperhomocysteinemia



Note: FA-def — subgroup of hypertensive patients with folic acid deficiency; FA-norm — subgroup of hypertensive patients with normal level of plasma folic acid * — statistically significant differences (P = 0.02) of glutathione reductase activity when comparing hypertensive patients with and without folic acid deficiency; ^ — statistically significant differences (P = 0.001) in the activity of glutathione reductase between patients with arterial hypertension with a deficiency of folic acid and the comparison group.

concentration of glucose, creatinine, transaminases and C-reactive protein, vitamin B 12 was determined using standard Roche kits for the Cobas Integra biochemical analyzer. Determination of total cholesterol concentration was performed using reagents from Abbott Clinical Chemistry. The concentration of FA in blood plasma was determined by the method of competitive immunochemiluminescence analysis on an Access 2 Immunoassay System (Beckman Coulter Inc., USA) enzyme immunoassay analyzer, which makes it possible to assess the total level of folates, including folic acid of exogenous origin and its endogenous active form 5- MTHF. In the presentation of the data, the term FA refers to the total folate level. Statistical analysis of the data obtained was carried out using the SPSS 21.0 for Windows software. The results were presented as the median and interquartile range Me (Q1-Q3). To test the hypothesis about the difference between the samples, we used nonparametric tests: in the case of two independent samples, Mann-Whitney, and for three independent samples, Kruskal-Wallace. At $p < 0.05$, the differences between the samples were considered significant. To assess correlations, Spearman's rank correlation coefficient was used.

Results and discussion

Studies have shown that in the general group of hypertensive patients, shifts in the metabolism of GSH in erythrocytes were expressed in a decrease in the concentration of GSH and a decrease in the activity of GPO and GR, both with normal and decreased levels of FA in the blood (Table 2), which indicates inhibition of the glutathione system specific to the OS. Depletion of the GSH resource can be explained by a decrease in the GR activity, which is confirmed by the positive correlation between the GR activity and the GSH concentration ($R = 0.559$; $p = 0.001$), revealed in the erythrocytes of hypertensive patients. The literature contains contradictory data on changes in the activity of the enzyme system of glutathione in hypertension. We observed both a decrease in the activity of GR and GPO [22, 23] and an increase in their activity during treatment with antihypertensive drugs [18, 24]. The decrease in the activity of these enzymes in hypertension was explained either by impaired expression or inactivation under OS [22]. Using the reference values of the concentration of FA in blood plasma indicated by the manufacturer of the test system, we divided the general group of patients with hypertension into subgroup 1 with normal (≥13.4 nM) and subgroup 2 with reduced (<13.4 nM) values. In the general group, GR activity positively correlated with the FA concentration ($R = 0.415$; $p = 0.001$). In subgroup 2 with a reduced level of FA, the GR activity was lower than in subgroup 1 (Table 2). The GSH level was also lower in subgroup 2 with FA deficiency, and these subgroups did not differ in the activity of GPO. Based on the data obtained, in 75 % of donors the level of tHcy did not exceed 10.9 μM, so we took it as the upper limit of the reference interval of the control group for determining the presence of HHcy. According to the data (Table 3), the values of the GSH level and GR activity in the subgroup of hypertensive patients with the presence of HHcy were lower than the values of these indicators in the subgroup without HHcy. Under physiological conditions, Hcy through the transsulfuration pathway through the intermediate

cystathionine is converted into the direct precursor of glutathione L-cysteine, which serves as an additional source for the synthesis of GSH. According to various estimates, approximately half of the intracellular pool of GSH in human liver cells originates from the Hcy-dependent transsulfuration pathway, which can be considered as an adaptive response to OS, leading to an increase in the rate of GSH synthesis in cells [27]. However, chronic OS can block this path from working. Contrary to expectations, an increase in tHcy concentration does not lead to an increase in GSH level, but to an even greater decrease due to oxidative modification of enzymes [27]. Glutathione-dependent GPO is inhibited by micromolar Hcy concentrations, both in vitro [28] and in vivo at the level of protein-enzyme translation [29]. Figure 1 shows that, despite the absence of HHcy, the GR activity was lower in hypertensive patients with FA deficiency than in hypertensive patients without deficiency. In the same group of hypertensive patients without HHcy, a positive correlation was found between GR and FA of moderate strength (R = 0.564; p = 0.03). This fact testifies to the special role of GR in the situation of FA deficiency in hypertension. The interrelation of FA, Hcy and glutathione metabolism was demonstrated when high doses of 5-MTHF (15 mg / day) were administered to patients with CVD and HHcy [30]. A decrease in tHcy levels was accompanied by an increase in AOA, by a decrease in the proportion of the oxidized form of glutathione and an increase in the concentration of GSH in the blood, which indicates the action of FA as a regulator of the redox potential of a cell associated with glutathione metabolism. The effect of FA may depend on both a decrease in its consumption and a decrease in the activity of the enzyme MTHFR, which is also associated with the levels of cobalamin and tHcy. Since 5-MTHF is sensitive to oxidation, oxidative degradation can become significant under environmental conditions. Thus, superoxide anion radicals cause the breakdown of folates [31], and increased catabolism of FA can lead to the development of a deficiency and an increase in the need for folates, despite adequate dietary intake. In general, the revealed decreased GR activity and decreased GSH level confirm the connection between impaired glutathione metabolism and the pathogenesis of hypertension. Many studies have shown the connection between hypertension and impaired glutathione metabolism [15–18]. It has been shown that in mononuclear cells of hypertensive patients, the level of GSH and the activity of the enzymes GR and GPO decrease [23]. A decrease in the activity of antioxidant enzymes in hypertension is explained by a decrease in the expression or inactivation of enzymes under conditions of OS [32]. Antihypertensive treatment reduces OS [33] and leads to an increase in GSH levels and a decrease in oxidized glutathione, as well as to a significant increase in the activity of enzymes involved in glutathione metabolism [18, 34]. Glutathione metabolism, deficiency of FA, HHcy, and DNA methylation are metabolically linked through one-carbon exchange and

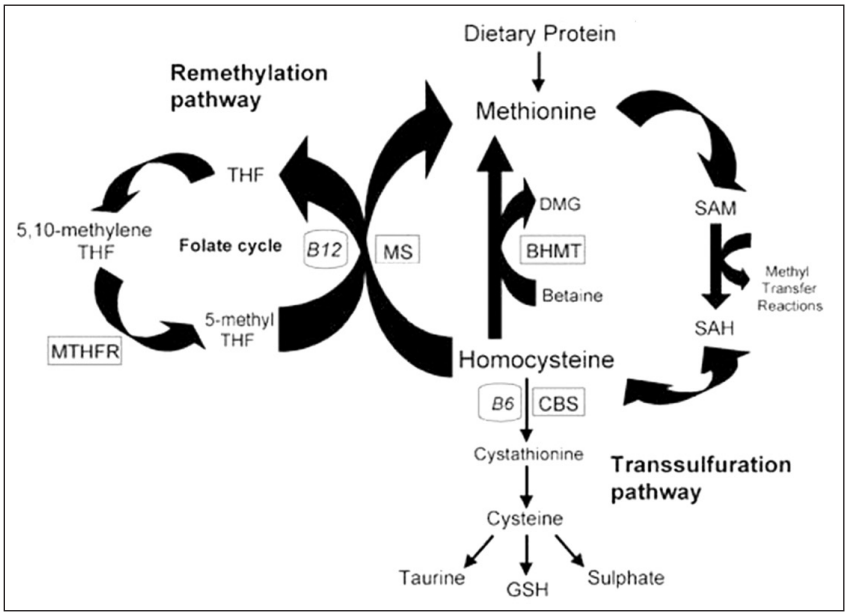
Table 3

PARAMETERS OF GLUTATHIONE METABOLISM IN PATIENTS WITH HYPERTENSION WITH FOLIC ACID DEFICIENCY DEPENDING ON THE PRESENCE OF HYPERHOMOCYSTEINEMIA

Parameter	Comparison group (N = 32)	Subgroup of patients htn without hhcy (N = 10)	Subgroup of patients htn with hhcy (N = 9)
GSH, μM/g Hb	3.3 (2.4–3.8)	1.9 (1.4–5.4)	1.5 (1.0–2.2) p* = 0.001 p** = 0.039
GR, U/g Hb	1.57 (1.23–2.06)	1.3 (0.83–2.0)	0.9 (0.6–1.1) p* = 0.001 p** = 0.002
GPO, U/g Hb	15.1 (10.9–18.3)	6.5 (5.8–8.9)	7.4(5.5–9.2)
tHcy, μmol/L	8.1 (6.5–10.9)	7.5 (6.4–8.6)	14.9 (13.1–24.9)
FA (nmol/L)	> 13.4	15.1 (12.4–21.8)	13.2 (9.9–16.9)

Note: P * is the level of statistical significance of differences when comparing subgroup indicators with control; P ** is the level of statistical significance of differences when comparing subgroup indicators with each other

Figure 2. Metabolic pathways linking folic acid, homocysteine and glutathione [35]



Note: THF — tetrahydrofolate; MTHFR — methylenetetrahydrofolate reductase; MS — methionine synthase; BHMT — betaine homocysteine methyltransferase; CBS — cystathionine β synthase; SAM — S-adenosylmethionine; SAH — S-adenosylhomocysteine.

transsulfuration (Fig. 2). Enzymes involved in DNA methylation, including DNA methyltransferases, can exhibit altered activity under conditions of insufficient glutathione cell defense system. In vitro studies show that depletion of GSH in cells leads to global DNA hypomethylation, possibly due to depletion of S-adenosylmethionine [35]. The interrelation between the GSH level and GR activity and FA deficiency revealed in our study indicates the involvement of glutathione in the development of functional folate deficiency in hypertension. Therefore, in patients with hypertension, it is recommended to monitor the parameters of glutathione metabolism and use FA in complex therapy in case of its functional deficiency. The feasibility of using FA in hypertension has been convincingly shown in a study among adults with hypertension in China, who did not have a history of stroke or myocardial infarction, and the combined use of enalapril and folic acid compared with enalapril alone significantly reduced the risk of first stroke and myocardial infarction [36]. These results are consistent with the benefits of folate use among hypertensive patients and low baseline folate levels [37] The question of whether FA supplementation should be recommended for secondary prevention of cardiovascular disease remains open. A search for new markers of latent functional FA deficiency and additional data from large-scale randomized trials are required.

Conclusion

The suppression of the glutathione-dependent system of erythrocytes is associated with a decrease in the level of GSH, the activity of GPO and GR in hypertension. Deficiency of FA and HHcy promote a shift in the redox potential of erythrocyte glutathione due to a decrease in its reduced form and a decrease in the activity of GR. Moreover, even a slight deficiency of folate increases the lack of GR activity, regardless of the level of tHcy. The parameter of GR activity in erythrocytes can be considered as a possible factor of functional FA deficiency in the absence of HHcy.

Conflict of interest

The authors declare no conflict of interest.

References

1. Pravenec M, Kozich V, Krijt J, Sokolová J, Zídek V, Landa V et al. Folate deficiency is associated with oxidative stress, increased blood pressure, and insulin resistance in spontaneously hypertensive rats. Am J Hypertens. 2013;26(1):135–140. doi:10.1093/ajh/hps015

2. Yi X, Zhou Y, Jiang D, Li X, Guo Y, Jiang X. Efficacy of folic acid supplementation on endothelial function and plasma homocysteine concentration in coronary artery disease: a meta-analysis of randomized controlled trials. Exp Ther Med. 2014;7(5):1100–1110. doi:10.3892/etm.2014.1553

3. Stanger O. Physiology of folic acid in health and disease. Curr Drug Metab. 2002;3(2):211–223. doi:10.2174/1389200024605163

4. Anguera MC, Suh JR, Ghandour H, Nasrallah IM, Selhub J, Stover PJ. Methylenetetrahydrofolate synthetase regulates folate turnover and accumulation. J Biol Chem. 2003;278(32):29856–29862. doi:10.1074/jbc.M302883200

5. An Y, Feng L, Zhang X, Wang Y, Wang Y, Tao L et al. Dietary intakes and biomarker patterns of folate, vitamin B_6, and vitamin B_{12} can be associated with cognitive impairment by hypermethylation of redox-related genes NUDT15 and TXNRD 1. Clin Epigenetics. 2019;11(1):139. doi:10.1186/s13148-019-0741-y

6. Cui S, Lv X, Li W, Li Z, Liu H, Gao Y et al. Folic acid modulates VPO1 DNA methylation levels and alleviates oxidative stress-induced apoptosis in vivo and in vitro. Redox Biol. 2018;19:81–91. doi:10.1016/j.redox.2018.08.005

7. Poltavtseva OV, Nesterov UI, Teplyakov AT. Homocysteinemia in patients with arterial hypertension and cerebrovascular complications. Sibirskiy Meditsinskiy Jurnal = Siberian Medical Journal. 2012;27(4):37–41. In Russian.

8. The evaluation of folate status using total homocysteine in hypertensive patients. Rossijskiy Meditsinskiy Zhurnal = Russian Medical Journal. 2019;25(3):158–165. In Russian.

9. Zhloba AA. Laboratory diagnosis of hyperhomocysteinemia. Klinico-laboratornyy Konsillium = Clinical Laboratory Consillium. 2009;26(1):49–60. In Russian.

10. Essouma M, Noubiap JN. Therapeutic potential of folic acid supplementation for cardiovascular disease prevention through homocysteine lowering and blockade in rheumatoid arthritis patients. Biomark Res. 2015;3:24. doi:10.1186/s40364-015-0049-9

11. Shen M, Tan H, Zhou S, Retnakaran R, Smith GN, Davidge ST et al. Serum folate shows an inverse association with blood pressure in a cohort of chinese women of childbearing age: A Cross-Sectional Study. PLoS One. 2016;11(5):e0155801. doi:10.1371/journal.pone.0155801

12. Verhaar MC, Stroes E, Rabelink TJ. Folates and cardiovascular disease. Arterioscler Thromb Vasc Biol. 2002;22(1):6–13. doi:10.1161/hq0102.102190

13. Wang Y, Jin Y, Wang Y, Li L, Liao Y, Zhang Y et al. The effect of folic acid in patients with cardiovascular disease: A systematic review and meta-analysis. Medicine (Baltimore). 2019;98(37):e17095. doi:10.1097/MD.0000000000001709512

14. Bunout D, Petermann M, Hirsch S, de la Maza P, Suazo M, Barrera G et al. Low serum folate but normal homocysteine levels in patients with atherosclerotic vascular disease and matched healthy controls. Nutrition. 2000;16(6):434–438. https://doi.org/10.1016/S 0899-9007(00)00289-6

15. Stanhewicz AE, Kenney WL. Role of folic acid in nitric oxide bioavailability and vascular endothelial function. Nutr Rev. 2017;75(1):61–70. doi:10.1093/nutrit/nuw053

16. Yuyun MF, Ng LL, Ng GA. Endothelial dysfunction, endothelial nitric oxide bioavailability, tetrahydrobiopterin, and 5-methyltetrahydrofolate in cardiovascular disease. Where are we with therapy? Microvasc Res. 2018;119:7–12. doi:10.1016/j.mvr.2018.03.012

17. Mikashinowich ZI, Nagornaya GJ, Kovalenko TD. The role of antioxidant enzymes in pathogenesis of arterial hypertension at teenagers. Med Herald South Russ. 2013;(3):60–62. doi:10.21886/2219-8075-2013-3-60-62. In Russian.

18. Montezano AC, Touyz RM. Molecular mechanisms of hypertension — reactive oxygen species and antioxidants: a basic science update for the clinician. Can J Cardiol. 2012;28(3):288–295. doi:10.1016/j.cjca.2012.01.017

19. Montezano AC, Touyz RM. Reactive oxygen species, vascular noxs, and hypertension: focus on translational and clinical research. Antioxid Redox Signal. 2014;20(1):16–182. doi:10.1089/ars.2013.5302

20. Rybka J, Kupczyk D, Kędziora-Kornatowska K, Motyl J, Czuczejko J, Szewczyk-Golec K et al. Glutathione-related antioxidant defense system in elderly patients treated for hypertension. Cardiovasc Toxicol. 2011;11(1):1–9. doi:10.1007/s12012-010-9096-5

21. Ballatori N, Krance SM, Notenboom SN, Shi S, Tieu K, Hammond CL. Glutathione dysregulation and the etiology and progression of human diseases. Biol Chem. 2009;390(3):191–214. doi:10.1515/BC.2009.033

22. Alexandrova LA, Mironova JA, Filippova NA, Trjofimov VI. Glutathione metabolism of erythrocytes in the paroxysmal nocturnal hemoglobinuria. Regionarnoe Krovoobrashchenie i Mikrotsirkulyatsiya = Regional Blood Circulation and Microcirculation. 2015;14(4):60–65. doi:10.24884/1682-6655-2015-14-4-60-65. In Russian.

23. Zhloba AA, Subbotina TF. Homocysteinylation score of high molecular weight plasma proteins. Amino Acids. 2014;46(4):893–899. doi:10.1007/s00726-013-1652-4

24. Chaves FJ, Mansego ML, Blesa S, Gonzalez-Albert V, Jimenez J, Tormos MC et al. Inadequate cytoplasmic antioxidant enzymes response contributes to the oxidative stress in human hypertension. Am J Hypertens. 2007;20(1):62–69. doi:10.1016/j.amjhyper.2006.06.006

25. Silva AP, Marinho C, Goncalves MC, Monteiro C, Laires MJ, Falcao LM et al. Decreased erythrocyte activity of methemoglobin and glutathione reductases may explain age-related high blood pressure. Rev Port Cardiol. 2010;29(3):403–412.

26. Grossman E. Does increased oxidative stress cause hypertension? Diabetes Care. 2008;31(Suppl. 2):185–189. doi:10.2337/dc08-s246

27. Mosharov E, Cranford MR, Banerjee R. The quantitatively important relationship between homocysteine metabolism and glutathione synthesis by the trassulfuration pathway and its regulation by redox changes. Biochemistry. 2000;39(42):13005–13011. doi:10.1021/bi001088w

28. Chen N, Liu Y, Greiner CD, Holtzman JL. Physiologic concentrations of homocysteine inhibit the human plasma GSH peroxidase that reduces organic hydroperoxides. J Lab Clin Med. 2000;136(1):58–65. doi:10.1067/mlc.2000.107692

29. Handy DE, Zhang Y, Loscalzo J. Homocysteine down-regulates cellular glutathione peroxidase (GPx1) by decreasing translation. J Biol Chem. 2005;280(16):15518–15525. doi:10.1074/jbc.M501452200

30. Caruso R, Campolo J, Sedda V, De Chiara B, Dellanoce C, Baudo F et al. Effect of homocysteine lowering by 5-methyltetrahydrofolate on redox status in hyperhomocysteinemia. J Cardiovasc Pharmacol. 2006;47(4):549–555. doi:10.1097/01.fjc.0000211748.16573.31

31. Shaw S, Jayatilleke E, Herbert V, Colman N. Cleavage of folates during ethanol metabolism. Biochem J. 1989;257(1):277–280. doi:10.1042/bj2570277

32. Wu G, Fang YZ, Yang S, Lupton JR, Turner ND. Glutathione metabolism and its implications for health. J Nutr. 2004;134(3):489–492. doi:10.1093/jn/134.3.489

33. Brain KL, Allison BJ, Niu Y, Cross CM, Itani N, Kane AD et al. Intervention against hypertension in the next generation programmed by developmental hypoxia. PLOS Biology. 2019;17(1): e2006552. doi:10.1371/journal.pbio.2006552

34. Taddei S, Virdis A, Ghiadoni L, Sudano I, Salvetti A. Antihypertensive drugs and reversing of endothelial dysfunction in hypertension. Curr Hypertens Rep. 2000;2(1):64–70. doi:10.1007/s11906-000-0061-8

35. Austin R, Lentz S, Werstuck, G. Role of hyperhomocysteinemia in endothelial dysfunction and atherothrombotic disease. Cell Death Differ. 2004;11(Suppl. 1): S 56–S 64. doi:10.1038/sj.cdd.4401451

36. Niedzwiecki MM, Hall MN, Liu X, Oka J, Harper KN, Slavkovich V et al. Blood glutathione redox status and global methylation of peripheral blood mononuclear cell DNA in Bangladeshi adults. Epigenetics. 2013;8(7):730–738. doi:10.4161/epi.25012

37. Mahajan AS, Babbar R, Kansal N, Agarwal SK, Ray PC. Antihypertensive and antioxidant action of amlodipine and vita-min C in patients of essential hypertension. J Clin Biochem Nutr. 2007;40(2):141–147. doi:10.3164/jcbn.40.141

38. Huo Y, Li J, Qin X, Huang Y, Wang X, Gottesman RF et al. Efficacy of folic acid therapy in primary prevention of stroke among adults with hypertension in China: The CSPPT Randomized Clinical Trial. J Am Med Assoc. 2015;313(13):1325–1335. doi:10.1001/jama.2015.2274

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