THE EFFECTS OF SUBCHRONIC METHIONINE OVERLOAD ADMINISTERED ALONE OR SIMULTANEOUSLY WITH L-CYSTEINE OR N-ACETYL-L-CYSTEINE ON BODY WEIGHT, HOMOCYSTEINE LEVELS AND BIOCHEMICAL PARAMETERS IN THE BLOOD OF MALE WISTAR RATS

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EFEKTI SUBHRONIČNOG OPTEREĆENJA METIONINOM SAMOSTALNO ILI U KOMBINACIJI SA L-CISTEINOM ILI N-ACETIL-L-CISTEINOM NA TELESNU MASU, VREDNOSTI UKUPNOG HOMOCISTEINA I BIOHEMIJSKE PARAMETRE U KRVI MUŽJAKA WISTAR PACOVA

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ABSTRACT

Hyperhomocysteinemia (HHC), both basal and after methionine load, may occur due to genetic disorders or deficiencies of nutrients that affect the remethylation or transsulphuration pathways during methionine metabolism. HHC is involved in the pathogenesis of many illnesses as a

SAŽETAK

Hiperhomocisteinemija (HHC), kako bazalna tako i nakon opterećenja metioninom, može nastati usled poremećaja genetskih ili nutricionih faktora u putevima remetilacije ili transsulfuracije tokom metabolizma metionina, uključena u patogenezi brojnih bolesti kako svojim prooksidantnim dejstvom,

ABBREVIATIONS

AHDL - direct high-density cholesterol;
ALB – albumin;
ALP - alkaline phosphatase;
ALT - alanine aminotransferase;
AMY – amylase;
AST - aspartate aminotransferase;
bw – body weight
BUN - blood urea nitrogen;
Ca – calcium;
Cl – chloride;
CHOL – cholesterol;
CREA – creatinine;
ECL -electrochemiluminescence immunoassay system;
GLUC – glucose;
GGT - gamma glutamyl transferase;
GSH – glutathione;
Hcy – homocysteine;
HHC – hyperhomocysteinemia;
IBCT - total iron binding capacity;
K – potassium;
L-cys - L-cysteine
LDL - low-density cholesterol;
MET - DL-methionine;
NAC - N-acetyl-L-cysteine;
Na – sodium;
RCRP - C-reactive protein;
ROS – reactive oxygen species;
TBI - total bilirubin;
TGL – triglyceride;
tHcy – total homocysteine;
TP - total protein;
URCA - uric acid

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result of its prooxidative effect and its impairment of antioxidative protection. The aim was to examine the effects of subchronic methionine overload on the body weight and standard biochemical parameters in rat serum and to examine whether simultaneous subchronic intraperotoneal administration of methionine alone or together with L-cysteine or N-acetyl-cysteine resulted in a change in the body weight and biochemical parameters in the rat serum. The research was conducted during a three-week period (male Wistar albino rats, n=36, body weight of approximately 160 g, age of 15-20 days), and the animals were divided into a control group and three experimental groups of 8-10 animals each: a) control group (0.9% sodium chloride 0.1-0.2 ml/day); b) methionine (0.8 mmol/kg/bw/day) (MET group); c) methionine (0.8 mmol/kg/bw/day) + L-cysteine (7 mg/kg/bw/day) (L-cys+MET group); and d) methionine (0.8 mmol/kg/bw/day) + N-acetyl-L-cysteine (50 mg/kg/bw/day) (NAC+MET group). In addition to the body weight monitoring, the levels of total homocysteine and the standard biochemical parameters in blood samples (plasma or serum) were determined. The results indicated that monitoring the homocysteine levels and standard biochemical parameters in blood could be used for analysis and could provide an excellent guideline for distinguishing between toxic and non-toxic doses of methionine intake, which may be meaningful for clinical applications.

Keywords: *methionine, L-cysteine, N-acetyl-L-cysteine, hyperhomocysteinemia, rat*

tako i oštećenjem antioksidativne zaštite. Cilj ovog rada bio da se ispitaju efekti subhroničnog opterećenja metioninom na promenu telesne mase i vrednosti standardnih biohemijskih parametara u serumu pacova i da li istovremena subhronična intraperotonealna primena metionina sa L-cisteinom ili N-acetil-L-cisteinom utiče na promenu telesne mase i vrednosti standardnih biohemijskih parametara u serumu pacova. Istraživanje je sprovedeno tokom perioda od 3 nedelje (mužjaci pacova soja Wistar albino n=36; tm oko160 g; starosti 15-20), pri čemu su životinje bile podeljene na jednu kontrolnu grupu i tri eksperimentalne grupe sa po 8-10 životinja u grupi, prema sledećoj shemi: a) kontrolna grupa (0,9% NaCl 0,1-0,2 ml/dan); b) metionin (0,8 mmol/kg/tm/dan) (MET grupa); metionin (0,8 mmol/kg/tm/dan) + L-cistein (7mg/kg/tm/dan) (L-cys+MET grupa); metionin (0,8 mmol/kg/tm/dan) + N-acetil-L-cistein (50 mg/kg/tm/dan) (NAC+MET grupa). Pored praćenja telesne mase životinja, u uzorcima krvi (plazma ili serum) određivane su vrednosti totalnog homocisteina i standardni biohemijski parametri. Na osnovu dobijenih rezultata zaključujemo da praćenje nivoa homocisteina i standardnih biohemijskih parametara u krvi predstavlja temelj u analizi i odličnu smernicu pri razlikovanju toksičnih od netoksičnih doza unetog metionina. Pored toga, rezultati ukazuju na antioksidacione i antiinfamatorne osobine N-acetilcisteina i L-cisteina što može imati važnu kliničku primenu.

Ključne reči: *metionin, L-cistein, N-acetil L-cistein, hiperhomocisteinemija, pacov*

INTRODUCTION

Homocysteine (Hcy) is a semi-essential amino acid that contains sulphur obtained from methionine. The necessary quantity of methionine in organisms is maintained by remethylation of homocysteine. The metabolism of methionine is the only known pathway of thiol amino acid production in organisms. Hyperhomocysteinemia (HHC), both basal and after methionine load, may occur due to genetic disorders or nutrition factors that affect the remethylation or transsulphuration pathways during methionine metabolism (1). The reactions in the methionine cycle also lead to formation of the tripeptide glutathione (GSH), with a considerable effect on the processes underlying the formation of free radicals and lipid peroxidation (2). The results of studies of chronic methionine overload suggest the presence of oxidative stress in the rat liver (3, 4). The hyperhomocysteinemia induced by methionine administration leads to increased production of free radicals and inflammation markers in macrophages of the mouse peritoneum (5). An *in vitro* and *in vivo* study on rats showed the alteration of oxidative stress parameters after the methionine administration (6), and hemosiderin accumulated in rat hepatocytes after a four-week methionine treatment (7). The occurrence of hyperhomocysteinemia was also reported in a population of patients with inflammatory bowel disease after an oral overload with methionine (8).

The literature data show that an increased level of homocysteine in blood is a risk factor for cardiovascular diseases, including ischemic cardiac disease and numerous vascular pathologies, chronic renal insufficiency and cerebrovascular disorders (9, 10). Many clinical studies showed that hyperhomocysteinemia is a risk factor for gastrointestinal diseases (11). The increased homocysteine level was shown in colon mucosa and plasma in patients with inflammatory bowel disease (12, 13).

Inflammatory bowel disease is a chronic, idiopathic inflammatory disease of the gastrointestinal tract, with examples including Crohn's disease and ulcerous colitis (14). The evolution of Crohn's disease might include intestinal and extra-intestinal complications, especially atherothrombotic events (15). Hyperhomocysteinemia has been recognized as one of numerous risk factors for the development of thrombotic conditions in patients with cardiovascular diseases (16). The metabolites of homocysteine and Hcy-related genes may be involved in the pathogenesis of ulcerous colitis (17) and coeliac disease (18). Hyperhomocysteinemia is related to inflammation and may be a risk factor for colorectal carcinoma, stomach carcinoma and carcinogenesis in patients with inflammatory bowel disease (19, 20, 21).

Therefore, hyperhomocysteinemia is probably involved in the pathogenesis of numerous diseases with both

its pro-oxidative effect and impairment of anti-oxidative protection. The superoxide dismutase, glutathione peroxidase and catalase enzymes are involved in protecting cells from the harmful effects of oxygen radicals (22, 23). The pathogenesis of numerous gastrointestinal diseases, such as peptic ulcer, gastrointestinal carcinoma and inflammatory bowel disease, partly results from oxidative stress (22). *Matte* et al. (24) noted the occurrence of oxidative stress in the liver of homocysteine-treated rats. In the conditions in which oxidative stress is a molecular cause of the disease, therapy with antioxidative agents, namely supplements with sulphur-containing amino acids (25), is increasingly mentioned in the literature as a possible solution.

N-acetyl-L-cysteine (NAC) is well known as an antioxidative agent that acts directly and/or by increasing intracellular GSH, especially in hepatic tissue. The administration of NAC has been reported to be helpful in many chronic clinical conditions, such as inflammatory bowel disease, obstructive lung disease and other lung diseases, systemic sclerosis, cystic fibrosis, humane immune deficiency syndrome, septic shock, diabetes and liver diseases (26, 27).

Methionine or α-amino γ-methylthio butyric acid, an essential amino acid, is a thiamine acid, as are cysteine and cystine. Methionine participates in protein synthesis, transmethylation reactions and the synthesis of the amino acids cysteine and cystine (28). Methionine is the final precursor of cysteine (one of the three amino acids that form GSH) (29).

The present knowledge about inflammatory bowel disease therapy has been provided by papers that show the protective, anti-inflammatory and antioxidative effects of N-acetyl-L-cysteine on experimentally induced colitis in rats (30, 31). The antioxidative capacity of N-acetyl-L-cysteine was also demonstrated in a model of chronic liver damage in rats (32). A study conducted in humans showed that the supplementation with N-acetyl-L-cysteine improved the oxidative status of patients after surgical treatments in the abdomen, which indicates the importance of the clinical application of this substance (33). Cysteine also has protective effects on the digestive tract, as shown in an experimental model of stomach ulcer in rats (34).

Due to the poor literature data, the aim of this paper was to investigate the effects of subchronic methionine overload on the body weight and standard biochemical parameters of rat serum. Additionally, the paper also examined whether simultaneous subchronic administration of methionine together with L-cysteine or N-acetyl-cysteine resulted in a change in the body weight or standard biochemical parameters in rat serum.

MATERIAL AND METHODS

Experimental animals

Male *Wistar albino* rats (n=36), with a body weight of approximately 160 g and age of 15-20 days at the beginning of the experiment (vivarium of Military Medical Academy in Belgrade), were used in the research on the subchronic administration of methionine. The rats were housed singly or in pairs in transparent Plexiglas cages with a wood-chip floor. Food and water were available *ad libitum*, and the ambient conditions were constant (temperature 21±2 °C; humidity 55±5 %; 12 h light-dark cycle with the light period beginning at 07:30 a.m.).

Experimental protocol

The research was conducted during a three-week period, for which the animals were divided into a control and three experimental groups, with 8-10 animals each. The animals in the experimental groups were administered an intraperitoneal daily dose of a specific substance, depending on the group, and the control group received the same quantity of 0.9% sodium chloride solution daily (35) according to the following protocol: a) control group (0.9% sodium chloride 0.1-0.2 ml/day) (n=10); b) methionine (0.8 mmol/kg/bw/day) (n=10) (MET group); c) methionine (0.8 mmol/kg/bw/day) + L-cysteine (7 mg/kg/bw/day) (n=8) (L-cys+MET group) (36); and d) methionine (0.8 mmol/kg/bw/day) + N-acetyl-L-cysteine (50 mg/kg/bw/day) (n=8) (NAC+MET group) (37). Prior to and during the research, all animals were permanently monitored, with tracking of the body weight of each animal in each group.

On the twenty-second day, the subchronic treatment with the appropriate amino acid(s) was complete; the animals were then sacrificed using a rat guillotine, and blood samples were taken. For this process, blood was collected through a glass funnel and placed in appropriate vacutainers (note: it is known that when this method is used, somewhat higher values of certain biochemical parameters are expected due to the severe mechanical trauma). After the collection, the samples remained at room temperature for 15 minutes and were then centrifuged (15 min x 3000 rpm) and analysed (plasma or serum). At the beginning of and after the experiment, the blood was analysed using the electrochemiluminescence method (ECL- electrochemiluminescence immunoassay system, ADVIA Centaur XP System, Siemens Healthcare GmbH, Erlangen, Germany); the range of reference values was Hcy< 15 μmol/l.

The serum biochemical parameters were measured using a spectrophotometric method and an indirect potentiometric method (sodium, potassium and chloride), while the C-reactive protein (CRP) level was determined by using the immunoturbidimetric method (PETIA). Commercial kits from Siemens Healthcare Diagnostics Ltd. (Frimley, Camberley UK) and an automatic analyser (Dimension Xpand, Siemens, Germany) were used.

Statistical analysis

For the statistical analysis of the experimental data, the parameters of descriptive statistics were used. For testing statistical significance after testing the normality of the

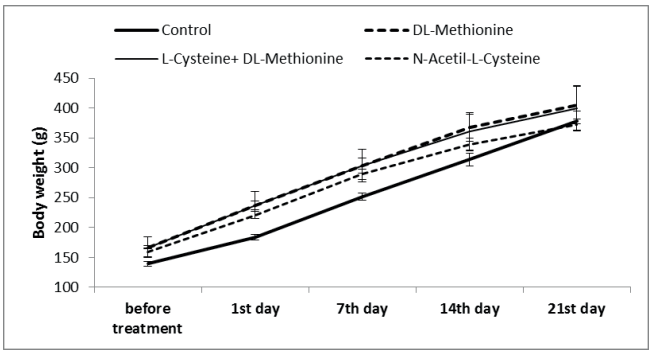

Figure 1. Changes of rat body weight in the control and experimental groups during the three-week experimental period of the intraperitoneal administration of tested substances (the results are shown as X±SE).

distribution, Student's T test (parameter test) was used for dependent and independent variables. Statistical calculations were performed using SPSS computer program (SPSS Inc. Chicago, SAD). P <0.005 was considered statistically significant.

RESULTS

Body weight (BW)

Body weight was the first parameter and was monitored during the entire experimental period (3 weeks). In the control group, which did not receive any of the examined substances, the body weight increased noticeably after each week of research, as in the experimental groups. The highest increase in body weight was found after the first and the third weeks of research. In the MET group, the increase in body weight was lower after the third week, while in the L-cys+MET group, a lower increase was found after both the second and third weeks of research. The same dynamics of this parameter was also found in the NAC+MET group (Fig. 1).

Table 1. Total homocysteine values (tHcy) in the blood of the examined rats (the results are presented as x±SE).

Groups	Total homocysteine (µmol/L)
Control	9.98±0.65
DL-Methionine	9.51±0.59
L-Cysteine + DL-Methionine	10.35±0.93
NAC + DL-Methionine	10.21±0.71

Table 2. Values from the statistical analysis for comparing total homocysteine levels in the blood for the various experimental groups. Values of p<0.05 were considered statistically significant.

Groups	Total homocysteine (µmol/L)
Control vs. DL-Methionine	0.549
Control A vs. L-Cysteine + DL-Methionine	0.741
Control vs. NAC + DL-Methionine	0.741
DL-Methionine vs. L-Cysteine + DL-Methionine	0.477
DL-Methionine vs. NAC + DL-Methionine	0.477

Total homocysteine (tHcy) in blood

The homocysteine levels in the group that received methionine alone (MET group) were lower than those in the groups that received L-cysteine + methionine (L-cys+MET) or N-acetylcysteine + methionine (NAC+MET). The values of this parameter were compared between the experimental and control groups, and differences in homocysteine levels were found but were not statistically significant (Tables 1 and 2)

Standard biochemical parameters

The glycaemia level was also monitored in this research. No statistically significant differences were found in the values of this parameter in any experimental groups compared to the control. In all experimental groups, a statistically significant decrease in the creatinine values was found, while the urea levels were significantly lower than in the control group. The comparison of experimental groups revealed a statistically significant difference in this parameter between the MET and L-cys+MET groups and also between the MET and NAC+MET groups. In contrast, compared to the control group, the NAC+MET group did not show any statistically significant differences in this parameter (Tables 3 and 4).

The lipid profile of animals was analysed in terms of the following parameters: cholesterol, triglycerides, LDL, and HDL. The cholesterol levels in the L-cys+MET group were considerably lower than those in the control group. The triglyceride levels were considerably lower in the L-cys+MET and MET groups, while the HDL levels were considerably lower in the MET group compared to the control. In contrast, the HDL levels were significantly higher in the L-cys+MET group than in the control group.

The electrolyte balance in the blood was monitored by recording the changes in the concentrations of Na, K, Cl and Ca. The sodium levels in the blood were significantly higher in all experimental groups than in the control, whereas there was no significant difference between the L-cys+MET and NAC+MET groups. The potassium levels were significantly lower in all groups compared to the control, but no difference between experimental groups was found. A statistically significant increase in the chloride and calcium levels was only found in the NAC+MET group compared to the control group, and a more significant increase was found when comparing the NAC+MET to the L-cys+MET group. The iron levels in the blood, the next parameter of interest, were only significantly lower in the NAC+MET group compared to the control.

Table 3. Values of standard biochemical parameters, shown as x ± SE.

PARAMETER/ GROUPS (x±SE)	CONTROL	DL-METHIONINE	L-CYSTEINE + DL-METHIONINE	N-ACETYL-L-CYSTEINE + DL-METHIONINE
GLUC	7.64 ± 0.12	6.66 ± 0.87	7.70 ± 0.21	7.61 ± 0.10
BUN	8.80 ± 0.64	6.24 ± 1.19	6.98 ± 0.28	7.60 ± 0.31
CREA	38.19 ± 1.25	29.71 ± 1.57	30.13 ± 1.19	32.25 ± 2.46
URCA	87.88 ± 4.37	47.50 ± 1.61	60.25 ± 4.09	59.75 ± 2.39
TBI	1.89 ± 0.09	2.50 ± 0.20	2.30 ± 0.21	2.14 ± 0.28
TP	63.63 ± 0.63	61.80 ± 0.57	66.50 ± 0.85	63.13 ± 0.77
ALB	13.56 ± 0.94	13.00 ± 0.30	13.13 ± 0.44	12.25 ± 0.37
CHOL	1.82 ± 0.03	1.72 ± 0.07	1.68 ± 0.05	1.64 ± 0.03
AHDL	1.59 ± 0.10	1.47 ± 0.04	1.63 ± 0.05	1.47 ± 0.03
TGL	0.71 ± 0.07	0.93 ± 0.05	1.05 ± 0.09	1.13 ± 0.11
Na	141.50 ± 0.29	145.60 ± 0.45	143.63 ± 0.63	143.00 ± 0.73
K	8.11 ± 0.16	6.76 ± 0.11	7.05 ± 0.22	7.54 ± 0.35
Cl	104.63 ± 0.24	104.70 ± 0.50	104.50 ± 0.27	105.25 ± 0.45
Ca	2.30 ± 0.12	2.06 ± 0.25	2.35 ± 0.02	2.47 ± 0.03
IRON	31.60 ± 1.79	28.02 ± 1.77	29.65 ± 1.89	31.59 ± 2.96
IBCT	112.44 ± 1.66	112.99 ± 2.62	114.25 ± 2.43	117.69 ± 3.08
AST	255.38 ± 13.63	202.20 ± 9.50	251.13 ± 18.88	241.25 ± 19.11
ALT	73.94 ± 8.20	55.40 ± 2.07	66.50 ± 3.76	67.25 ± 4.70
ALP	285.50 ± 12.50	253.60 ± 13.14	277.38 ± 14.74	298.50 ± 12.64
GGT	6.00 ± 0.35	6.00 ± 0.15	5.63 ± 0.18	5.50 ± 0.19
AMY	926.75 ± 77.34	1169.17 ± 48.97	1228.38 ± 50.10	1275.25 ± 40.02
RCRP	0.20 ± 0.03	0.80 ± 0.12	0.90 ± 0.13	0.90 ± 0.09

The liver and pancreas function was evaluated through the enzymes ALT, AST, γGT, ALP and AMY. The AST level was only significantly lower when comparing the MET group to the control, while the ALT level was significantly lower in the MET group compared to the L-cys+MET group. The ALP level was significantly lower in the MET group compared to the control and NAC+MET groups. The γGT levels did not significantly change in any of the groups, while the serum amylase levels were significantly higher in the MET and L-cys+MET groups compared to the control.

The CRP levels were significantly higher in all experimental groups compared to the control, but the differences were not significant for comparisons between experimental groups (Table 3). Table 4 shows the statistical values for the comparisons of the standard biochemical parameters between the different experimental groups.

DISCUSSION

The aim of the research was to evaluate the effects of subchronic methionine overload on body weight, the standard biochemical parameters in serum and plasma and, especially, the homocysteine level in rat blood. Additionally, the effects of simultaneous subchronic administration of methionine with L-cysteine or N-acetyl-L-cysteine on the body weight and the levels of standard biochemical parameters in the rat serum were examined.

Table 4. The statistical values for the comparisons of the standard biochemical parameters between the different experimental groups.

PARAMETER/ GROUPS	GLUC	BUN	CREA	URCA	TBI	TP	ALB	CHOL	AHDL	TGL	NA	K	CL	CA	IRON	IBCT	AST	ALT	ALP	GGT	AMY	RCRP
Control vs. DL-Methionine	.232	.008	.003	.000	.014	.038	.008	.179	.008	.015	.000	.000	.828	.854	.329	.712	.029	.051	.126	.255	.055	.001
Control vs. L-Cysteine + DL-Methionine	.538	.001	.000	.001	.021	.016	.036	.020	.500	.007	.008	.003	.649	.602	.501	.462	1.000	.854	.854	.639	.017	.000
Control vs. NAC + DL-Methionine	.951	.012	.031	.001	.110	.756	.003	.002	.009	.008	.108	.118	.183	.358	.903	.098	.520	.927	.297	.322	.011	.000
DL-Methionine vs. L-Cysteine + DL-Methionine	.228	.386	.861	.010	.261	.003	.781	.824	.045	.424	.024	.181	.708	.229	.756	.859	.051	.045	.197	.126	.302	.809
DL-Methionine vs. NAC + DL-Methionine	.334	.962	.450	.003	.166	.138	.136	.563	.563	.131	.009	.090	.465	.230	.398	.328	.168	.056	.021	.053	.071	.557
L-Cysteine + DL-Methionine vs. NAC + DL-Methionine	.526	.226	.598	.793	.708	.011	.145	.563	.031	.753	.520	.493	.154	.035	.431	.345	.529	.958	.345	.626	.462	.874

Methionine, which is an essential and thiol amino acid, is necessary for muscular contractions, haemoglobin synthesis, cholesterol and fat degradation (38). Hence, methionine participates in numerous biochemical processes in living organisms; its role in breaking down fat depots, synthesizing creatinine and increasing body weight is interesting (39). The intraperitoneal administration of methionine, i.e., its isomer DL-methionine, did not lead to a statistically significant increase in body weight that was proportional to time compared to the values in the control group. *Kluge* et al. examined the effects of DL-methionine on the growth of ducks during a 3-week period and concluded that the group of animals that received methionine in their food experienced an increase in body weight and growth without statistical significance and without improvement in physical performance, which is consistent with our results (40). However, recent experimental and clinical studies increasingly report that NAC is a potential therapeutic agent. *Elshorbagy* et al. examined the effect of sulphur amino acids (NAC, cysteine) on the prevention of obesity in rats. The animals received a methionine-restricted diet and were treated with the mentioned acids for 12 weeks; the results suggest that NAC and cysteine had significant effects with a decrease in body weight, primarily in the adipose tissue. Thus, the changes in body weight in the rats in our research can be explained by the presence of methionine during the entire experimental period, while NAC and L-cysteine probably blocked the capacity of methionine to prevent obesity, perhaps through the stearoyl-coenzyme A desaturase-1 enzyme (41, 42). However, further research is necessary to confirm this finding due to the considerable variations in the results of previous studies. These differences are primarily related to the variety in the experimental models (type of model, duration of experiment, dose, and form of the examined substance) and the differences in the sulphur amino acid administration procedures because the effects (e.g., NAC effects) significantly differ between intravenous and oral intake (43).

In addition to methionine and cysteine, homocysteine is a sulphur acid. Its modulatory effects are increasingly reported, and Hcy is considered a new risk factor, i.e., a damage marker. Numerous factors, including supplementation with sulphur amino acids, influence the homocysteine level in blood (44).

All compounds of this type have a free sulphur group and are capable of forming disulphides in plasma. Through this type of reaction with homocysteine, sulphur amino acids decrease the homocysteine concentration. However, in our study, the homocysteine level in blood only decreased in the group that received methionine alone. In contrast, when administered with L-cysteine or NAC, methionine did not decrease the Hcy level but instead increased it. In an experimental study, a 6-week methionine diet (1%) led to an increase in the homocysteine level in blood as a result of remethylation and transsulphuration processes (45), which conflicts with the findings in our study. However, chronic methionine overload is very toxic to various tissues and organs (46), thus increasing oxidative stress, thus stimulating adaptive reactions, i.e., compensatory responses. Such oxidative stress is correlated with the homocysteine level in blood; therefore, high homocysteine levels are expected when large doses of methionine are administered. This prediction indirectly agrees with our results because we used much less toxic doses in a shorter period than described in the mentioned study.

To further elucidate the subchronic effects of DL-methionine during this research, standard biochemical parameters were monitored (Table 1) and determined from blood samples (plasma or serum).

When ROS production is high, homeostasis is disturbed, leading to oxidative stress, which has a key role in the development of liver and other chronic diseases (5, 6). Oxidative stress generates liver damage by causing changes in lipids, proteins and DNK molecules and even more importantly, by modifying pathways that control normal biological functions. Moreover, the systemic oxidative stress that develops after liver disease may also lead to damage in other organs, such as the brain and kidneys. Consequently, homeostasis of the whole organism was monitored through biochemical parameters that are primarily associated with the biological processes in the liver, kidneys, pancreas and bowels. Due to the key role of oxidative stress in diseases of the liver and other organs, potential antioxidants, NAC and L-cysteine were also tested (27).

In our research, methionine alone or in combination with NAC and L-cysteine did not significantly influence the glycaemia levels. *Cole* et al. studied the effect of hyperglycaemia on the homocysteine and S-adenosyl-methionine (SAM) levels in the blood and plasma, and they showed that hyperglycaemia was correlated with the increased and decreased level of homocysteine (47).

These results are consistent with ours, due to the obvious decrease in Hcy levels and glycaemia in the DL-treated group. In the other groups, the increase in glycaemia was proportional to the increase in the Hcy level in the blood, probably as a result of lipid peroxidation after the oxidative stress. Both cysteine and NAC, together with other compounds that contain a cysteine group, participate in the regulation of the production of insulin and the maintenance of blood glycose levels, thus decreasing glyco-oxidation (48). Our study did not reveal statistically significant changes in this parameter after the administration of NAC and L-cysteine.

Impaired liver function is mostly shown by the change in "liver" parameters. Therefore, better knowledge of the changes in the pattern of serum parameters related to morphological and functional liver status might contribute to better treatment of diseases of the liver and other organs, thus making it easier to select optimal therapeutic modalities. In our study, the cholesterol and triglyceride levels were significantly lower than those in control group due to the effect of NAC and L-cysteine. This finding confirms the antioxidative capacity of these compounds because N-acetylcysteine is a glutathione prodrug that protects the liver from hepatic steatosis by

limiting the production of reactive oxygen species (6,7). Methionine removes metabolic waste products from the liver and can thus decrease the risk of hepatic and arterial steatosis. Methionine is important for the health of the liver and its detoxification and normal function. With the help of choline and inositol, methionine prevents fat accumulation in the liver. In our research, methionine also led to a decrease in hepatic lipid levels, although the decrease was not significant, possibly due to the shorter administration period compared to other studies (49). When compared to cysteine in another study, methionine inhibited the accumulation of fat to a higher degree; this result was also confirmed by our findings (50).

The administration of DL-methionine significantly reduced the parameters of hepatocellular and cholestatic liver damage, while the administration of NAC and L-cysteine caused a less significant decrease. In contrast, *Early* et al. investigated the effect of high concentrations of sulphur amino acids (DL-methionine, L-cystine) on the function and morphology of rat liver and concluded that an overload with sulphur amino acids during a short time resulted in liver atrophy and damage (51). Hence, the antioxidative properties of methionine, NAC and L-cysteine are probably dose dependent, i.e., only occur when lower doses are administered.

The degree of lipid peroxidation greatly depends on the degree of antioxidative protection in the organism at the moment when free radical production increases. When it occurs, lipid peroxidation changes the fluidity and permeability of the cell membrane, which results in electrolyte transport disorders, changes in protein content and modified functioning of organelles. *Roediger* compared the effects of two sulphur sources, inorganic NaHS and the amino acid L-methionine, on the redox status in rats to determine the mechanism underlying the development of ulcerous colitis to elucidate possible treatments. He concluded that L-methionine considerably decreased the beta-oxidation caused by NaHS in the colon and that exogenous methionine strongly influenced the quantity of SAM, which decreased the quantity of disulphide (52).

As a mechanism in the development of disease, a disruption of redox balance in favour of ROS occurs in many inflammatory conditions, primarily in the gastrointestinal tract (ulcerous colitis and Crohn's disease) (53). Proteins are oxidized in inflammatory diseases; thus, cell apoptosis occurs as a consequence of the cell exposure to a large quantity of ROS. The parameters of inflammation during the administration of three different amino acids were monitored in this paper, and no statistically significant change in CRP was found between these treatments and the control group. However, it is interesting that inflammatory parameters can be simultaneously monitored with homocysteine levels. In their study, *Panq* et al. reported that CRP directly participates in the occurrence and progression of atherosclerosis and that the homocysteine levels were correlated with the expression of CRP, whereby the administration of NAC decreased the expression of C-reactive protein. These data are interesting; they will certainly expand the perspectives in the domain of the prevention, diagnostics and therapy of inflammatory diseases (54).

The roles of iron in the metabolism of homocysteine have been described in the literature. Iron (Fe) catalyses the formation of Hcy from methionine, S-adenosylhomocysteine and cystathionine, thus increasing the circulating tHcy levels. Furthermore, free Fe catalyses the production of free oxygen radicals and the oxidation of small density lipoproteins, which is a known risk factor for vascular damage (55). The iron levels in blood were monitored in all groups. A significantly lower level of this parameter was found only in the NAC+MET group, while the tendency for increased iron levels was observed in the groups where the homocysteine levels were proportionally higher, which is a very important fact. Accordingly, *Lee* et al. noted that the increased values of iron in blood were related to mitochondrial dysfunction, which might result in increased ROS production; therefore, in the conditions of iron overload, N-acetylcysteine could be used as an antioxidant with the capacity to reduce ROS (56). This knowledge of the significance of Fe as a diagnostic and prognostic parameter, especially in conjunction with other biochemical parameters, is thus of great importance given that hyperhomocysteinemia is increasingly described in the literature as an independent risk factor for cardiovascular diseases.

CONCLUSIONS

The obtained results suggest that monitoring homocysteine levels and standard biochemical parameters in blood is crucial for analysis and provides an excellent guideline for distinguishing between toxic and non-toxic doses of methionine intake. Hence, homocysteine is a unique and reliable surrogate marker for evaluating the progression of metabolic disorders. In addition, supplementation with N-acetylcysteine and L-cysteine can have potential clinical applications, which needs to be confirmed by further clinical investigations.

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