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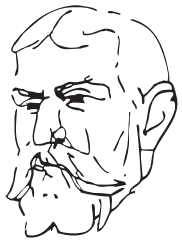

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CONTENTS

ORIGINAL ARTICLE

THE EFFECT OF S-ADEMETIONINE ON PLASMA CITRULLINE LEVEL DURING CHEMOTHERAPY-INDUCED OXIDATIVE STRESS IN PATIENTS WITH CHRONIC LYMPHOPROLIFERATIVE DISORDERS

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ABSTRACT

The aim: To investigate the effect of S-ademetionine on plasma citrulline level in patients with chronic lymphoproliferative disorders (CLPD) during chemotherapy-induced oxidative stress.

Materials and methods: 25 patients with CLPD were examined. Examinations were conducted twice: before chemotherapy (CT) and after 3 courses of CT. Several biochemical markers in the blood were determined: the activity of catalase, the level of plasma citrulline, the concentration of N-acetylneuraminic acid (NANA) and the concentration of substances that form a trimethine complex (TBARS) with 2-thiobarbituric acid. Patients were divided into groups: I (n=14) – patients who underwent only CT; II (n=16) – patients who during CT received S-ademetionine, at a dose of 1000 mg/day intravenously for 10 days, then 500 mg twice a day for 20 days. III (n=20) –the control group of 20 practically healthy individuals.

Results: Patients in both groups with CLPD had pre-existed mucosal injury that was characterized by 1.25 (p=0.0025) and 1.26 times (p=0.006) higher blood NANA concentration compared to the control group. The conduction of CT was associated with enterocytes dysfunction, which was characterized by 1,66 times (p=0,0002) lower plasma citrulline level in patients of group I compared to the initial examination. The infusion of S-ademetionine attenuated intestinal dysfunction that was associated with 1,23 times (p=0,0005) higher blood citrulline level after the CT as compared to group I.

Conclusions: The infusion of S-ademetionine as adjuvant treatment in patients with CLPD provided effective prophylaxis of intestinal injury that was associated with higher blood citrulline level after the conduction of CT.

KEY WORDS: S-ademetionine, mucositis, oxidative stress, citrulline

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INTRODUCTION

The major treatment option in patients with chronic lymphoproliferative disorders is chemotherapy (CLPD). The major concept in chemotherapy (CT) is to provide the infusion of chemotherapeutic drugs according to the dose-effectiveness principle. According to this principle, CT agents should be prescribed strictly according to the current standards of treatment provided by modern guidelines. At the same time infusion of CT agents in high doses is associated with a high risk of chemotherapy-induced injury of multiple organs and systems throughout the body. Nowadays, it is determined that many CT agents can cause inflammation and dysfunction of the entire gastrointestinal tract (GIT) [1].

The mucosal injury of GIT during CT is caused by the detrimental effect of CT agents on all rapidly proliferating cells. According to the current concepts, chemotherapy-induced mucositis can be divided into 2 groups: oral mucositis and intestinal mucositis. Most of the researchers dedicate their studies to oral mucositis, mostly because patients with oncological malignancies usually have numerous contraindications that make impossible the invasive assessment of small and large intestines [2]. Because of the impossibility to assess the status of small and large intestines by invasive methods, the diagnosis of intestinal mucositis requires a complex assessment of clinical symptoms simultaneously with an evaluation of specific intestinal biomarkers [3].

The state of GIT mucous membrane and chemotherapy-induced injury of the mucous membrane can be assessed by the level of N-acetylneuraminic acid (NANA) in the blood [4].

Currently, blood citrulline level is considered one of the most promising potential biomarkers in the assessment of intestinal injury. Citrulline is the amino acid, that is produced practically exclusively by enterocytes, thereby the assessment of blood citrulline level can be used in clinical management as a marker of enterocytes functional state [5].

Intestinal mucositis (IM) is one of the most common complications during the conduction of CT [6]. According to modern concepts, the resistance of the intestinal mucosal barrier is provided by 3 lines of defense: intestinal microbiota, mucosal layer and epithelium. One of the most important GIT barrier components – is the mucosal layer that provides dynamic and reciprocal interactions with various barrier components including intestinal microbi-

ota. Thus, the mucous layer provides physical, biochemical and biological defense against various aggressive factors and bacteria [7].

Among the agents, that are widely used in the treatment of CLPD is cyclophosphamide. Cyclophosphamide injection is associated with dangerous side effects such as bone marrow suppression simultaneously with suppression of the immune system, which results in the injury of the entire GIT. Dysfunction of the intestinal barrier is also considered one of the major side effects of cyclophosphamide. Cyclophosphamide causes disruption of the functional integrity and functional capacity of the intestinal mucosal layer, which affects tolerability to CT agents and prognosis in patients that ongoing CT [8].

In patients with CLPD the injections of Vincristine can also cause GIT injury. Vincristine causes disruption of GIT motility that clinically manifests with nausea, anorexia and paralytic intestinal obstruction [9].

The injection of Doxorubicin is also strongly associated with GIT injury, which manifests with clinical symptoms such as nausea and mucositis [10].

One of the major pathological mechanisms of CT-induced intestinal mucositis is oxidative stress [1]. Oxidative stress develops when the production of reactive oxygen species (ROS) prevails over the capacity of antioxidant defense to eliminate them [11].

It was determined that exactly the cytotoxic properties of CT agents are strongly associated with oxidative stress [12]. But also, the production of ROS during CT, potentiates various processes, that lead to cellular death including apoptosis [13].

From this perspective, the prophylaxis of CT-induced complications should include the agents that potentiate antioxidant defense. According to several clinical types of research in oncological practice to prevent chemotherapy-induced reactions, S-ademetionine can be used, as entroprotective agent [14]. Due to a systemic effect, S-ademetionine potentially can prevent oxidative injury to multiple organs and systems throughout the body, including the intestine.

It should be noticed, that currently, the application of S-ademetionine in oncological practice remains largely unstudied.

THE AIM

The aim is to investigate the effect of S-ademetionine on plasma citrulline level in patients with chronic lymphoproliferative disorders during chemotherapy-induced oxidative stress.

MATERIALS AND METHODS

24 patients with CLPD were studied: 15 (75%) patients with B-cell CLL and 10 (25%) patients with Small B-cell NHL. All patients were treated in the Hematology Department of M.V. Sklifosovsky Poltava Regional Clinical Hospital during 2018–2021 years. 7 (28 %) females and 18 (72 %) males, ages 30-76. The criteria of inclusion were the progression of B cell CLL, Small B-cell NHL; ECOG performance status – I-II, and Karnofsky Performance Status – 60-80%. All patients were diagnosed with B-CLL, Small B-cell NHL, indications for CT were determined, CT was appointed according to the current standards of treatment of patients with oncohematological malignancies with acute and chronic hemoblastosis, according to the order № 647 of the Ministry of Health of Ukraine since 30.07.2010, European Society for Medical oncology [15]. All patients whose CT has been complicated by diarrhea syndrome were enrolled into the study. The examinations were conducted twice: before CT and after 3 courses of CT. Patients were analysed for the following biochemical markers: the severity of the oxidative stress, which was determined by the concentration of substances, that form a trimethine complex (TBARS) with 2-thiobarbituric acid and the state of the antioxidant system (AOS) was analysed by catalase activity. Resistance of the mucous barrier of the intestine was determined by the concentration of NANA in the blood [16]. The enterocytes functional state was determined by the concentration of the citrulline in the plasma [17].

All patients received CT according to current standards, specifically, the following combinations were used: BR (bendamustine, rituximab), FCR (fludarabine, cyclophosphamide, rituximab), R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, prednisolone).

Depending on the inclusion of S-ademetionine as an adjuvant treatment, patients were divided into groups:

I (n=13) – patients with B-cell CLL and Small B-cell NHL, who received only CT.

II (n=14) – patients with B-cell CLL and Small B-cell NHL, who received S-ademetionine at a dose of 1000 mg/day intravenously for 10 days, then 500 mg twice a day for 20 days.

III (n=20) – the control group of 20 practically healthy individuals (9 (45 %) females and 11 (55 %) males, ages 22–26).

GraphPad Prism version 5.00 (GraphPad Software, Inc., San Diego, CA, USA) was applied for statistical analysis software. Normally, distributed data were expressed as mean ± standard deviation. Students criteria were used for normally distributed data. Non-parametric Wilcoxon-Mann-Whitney test was used for the analysis of unevenly distributed data. Spearman's rank correlation coefficients were used to assess the correlations between the results. For studies comparing prevalence between two groups, p value of < 0.05 was considered statistically significant.

RESULTS

On the initial examination in patients with CLPD of groups I and II the level of NANA was in 1.25 times (p=0,0025) and 1.26 times (p=0,006) respectively higher as compared to the control group (table I, II). A significant increase in NANA can be a sign of the detrimental effect of the oncohematological process on GIT mucose membrane.

In patients of both group I and group II on initial examination, the concentration of TBARS in the blood was in 1.39 times (p=0.0479) and 1,3 times (p=0.0122) respec-

Table I. Biochemical values in patients of the I group before and after treatment, M±m

Values	I (n=12)		III (n=20)
	V1	V2	
NANA, μmol/g	2,41±0,19* 95%CI 2,30-2,53	2,99±0,19& 95%CI 2,88- 3,11	1,93±0,19# 95%CI 1,845-2,029
Catalase, μmol/g	13,47±2,03* 95%CI 12,24-14,70	11,94± 2,23 95%CI 10,59- 13,29	12,98±2,19 95%CI 11,95-14,01
TBARS, nkat/g	1,56 ± 0,14* 95%CI 1,478-1,65	2,00± 0,16& 95%CI 1,90- 2,10	1,13 ± 0,55# 95%CI 0,87- 1,39
Plasma citrulline, μmol/l	47,39±3,86* 95%CI 45,06-49,73	28,58± 3,41& 95%CI 26,51- 30,64	57,20±9,88# 95%CI 52,70-61,70

Note: V1 – the first examination, V2 – the second examination, * - statistically significant difference between V1 and V2 values in patients of the I group (p<0,05), &- statistically significant difference between V1 values in patients of the I group and the III group (p<0,05), # - statistically significant difference between V2 values in patients of the I group and the III group (p<0,05).

Table II. Biochemical values in patients of the II group before and after treatment, M±m

Values	I (n=12)		III (n=20)
	V1	V2	
NANA, μmol/g	2,44±0,26 95%CI 2,27-2,60	2,63± 5,81& 95%CI 2,54-2,72	1,93±0,19# 95%CI 1,84-2,02
Catalase, μmol/g	12,92±2,25* 95%CI 11,49-14,35	11,80± 1,68 95%CI 10,73- 12,87	12,98±2,19 95%CI 11,95-14,01
TBARS, nkat/g	1,47±0,22 95%CI 1,33-1,61	1,43± 0,14& 95%CI 1,34-1,52	1,13 ± 0,55# 95%CI 0,87- 1,39
Plasma citrulline, μmol/l	49,85±5,22* 95%CI 46,53-53,17	35,22± 2,08 95%CI 33,89- 36,55	57,20±9,88# 95%CI 52,70-61,70

Note: V1 – the first examination, V2 – the second examination, * - statistically significant difference between V1 and V2 values in patients of the I group (p<0,05), &- statistically significant difference between V1 values in patients of the I group and the III group (p<0,05), # - statistically significant difference between V2 values in patients of the I group and the III group

tively higher compared to the control group (table I, II), which can be a sign of oxidative stress that was induced by the oncological process.

Simultaneously the citrulline concentration in the blood in patients of group I was 1.2 times (p=0.0161) lower compared to the control group. This can be explained by moderate functional dysfunction of enterocytes.

After the third course of CT in patients of group I, that underwent CT without the inclusion of S-ademetionine for adjuvant treatment, the level of NANA was in 1.24 times (p=0.0025) higher compared to the initial examination and 1,55 times (p=0.0017) higher (p=0.0017) compared to the control group (table I, II).

In patients of group II, that received S-ademetionine for adjuvant treatment, after the third course of CT the level of NANA was in 1.36 (p=0,0025) times higher compared to the control group (table I, II). The elevation of NANA in the blood can be a marker of chemotherapy-induced mucosal injury of the intestine. It should be noted that in patients of group II that received S-ademetionine the level of NANA was in 1.14 times (p=0,0059) lower compared to group I (table I, II), which can be a sign of mild enteroprotective effect of S-ademetionine.

After the secondary examination in patients of groups I and II the activity of catalase in the blood was in 1.13 times (p=0.0144) and in 1.1 times (p=0.0106), respectively lower compared to the initial examination (table I, II). That can be explained by the depletion of the antioxidant system during the CT.

During the conduction of CT in patients of group I the level of TBARS in the blood was in 1.28 times (p=0.0002) higher than on the initial examination (table I, II). The elevation of TBARS concentration in the blood can be explained by the development of chemotherapy-induced oxidative stress. The infusion of S-ademetionine as an adjuvant treatment to patients of group II, caused a decrease in 1.4 times (p=0.0005) of the TBARS concentration in the blood (table I, II), which can be a marker of a decrease in the severity of oxidative stress.

The conduction of the CT was associated with a decrease of citrulline in blood concentration in patients of both groups I and II, in 1.66 times (p=0.0002) and 1.42 times (p=0.0025) respectively, compared to the initial examination (table I, II). The decrease in citrulline level can be a marker of the enterocyte dysfunction, that was caused by the toxicity of CT. It should be noted that on the second examination, in patients of group II that received S-ademetionine as adjuvant treatment blood citrulline level was in 1.23 times (p=0,0005) higher compared to group I, that received only CT (table I, II). The possible reason for a

higher citrulline level in group II, can be enteroprotective effect of S-ademetionine.

DISCUSSION

In patients with CLPD pre-existed mucosal injury was determined, which was characterized by high blood NANA concentration. Pre-existed mucosal injury in oncohematological patients can be caused by the detrimental effect of malignancy itself that initially disrupt the mucosal functional state, which potentially can result in morphological injuries such as erosions or ulcers. One of the major mechanisms of mucosal injury in patients with CLPD is oxidative stress [6]. Oncological malignancy creates a prooxidative state, that in patients with CLPD was characterized by high TBARS levels in the blood [11]. Pre-existed mucosal injury and prooxidative state combined can significantly affect the functional state of enterocytes. The level of blood citrulline is used to assess the functional state of enterocytes. Because in several cases it's impossible to assess the status of small and large intestines by invasive methods, biomarkers such as citrulline can be the optimal choice [3]. Blood citrulline is produced practically exclusively by enterocytes, thus decrease in blood citrulline level can be a marker of a moderate functional dysfunction of enterocytes [5]. It was determined that patients with CLPD have decreased blood citrulline level. In patients with CLPD the pre-existed enterocytes dysfunction most probably was caused by malignancy-induced oxidative stress and was associated with mucosal injury.

The conduction of CT was associated with a further increase in blood NANA concentration, which can be a sign of aggravation of the pre-existing mucosal injury. Most probably mucosal injury was caused by CT-induced oxidative stress because most agents that are used in CT can induce inflammation and dysfunction of the entire GIT [1]. The infusion of CT agents creates a significant prooxidative state. The prooxidative state was created by an increase in reactive oxygen species (ROS) production, which was determined by the elevation of blood TBARS concentration, simultaneously with depletion of the antioxidant system, which was determined by a decrease in blood catalase concentration. The combination of an increase in ROS production with a decrease in ROS elimination causes severe oxidative stress that with time results in the oxidative injury of multiple cells in the human body, including enterocytes [11]. Oxidative injury of enterocytes caused severe functional dysfunction of enterocytes that was determined by decrease in blood citrulline level.

In patients of group II that received S-ademetionine as adjuvant treatment after CT the levels of NANA and TBARS were significantly lower compared to group I, which can be a marker of a milder mucosal and oxidative injury, respectively. Simultaneously in patients that received S-ademetionine the level of citrulline was significantly higher as compared to group I, which can be explained by the enteroprotective effect of S-ademetionine.

Thus, the infusion of S-ademetionine in patients of group II as adjuvant treatment provided the effective prophylaxis of chemotherapy-induced intestinal injury, due to its antioxidant and enteroprotective features.

CONCLUSIONS

Currently, the only treatment option for patients with CLPD is CT. However, many cytostatic drugs that are used in the treatment of CLPD have a significant enterotoxicity. Because sometimes in patients with CLPD, especially during CT, it's impossible to assess the status of small and large intestines the evaluation of specific intestinal biomarkers such as citrulline can be the optimal choice. The infusion of S-ademetionine as adjuvant treatment in patients with CLPD provided effective prophylaxis of intestinal injury that was associated with higher blood citrulline level after the conduction of CT. The enteroprotective effect of S-ademetionine most probably is provided by its potent antioxidant properties. Because the major pathogenic mechanism of mucosal injury in patients that receive CT agents is oxidative stress, attenuation of oxidative injury by S-ademetionine antioxidant properties can provide effective prophylaxis of chemotherapy-induced enterocytes dysfunction.

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