PHARMACIA

Pharmacia 69(2): 509–516
DOI 10.3897/pharmacia.69.e82596

Research Article

Therapy of post-COVID-19 syndrome: improving the efficiency and safety of basic metabolic drug treatment with tiazotic acid (thiotriazoline)

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Received 21 February 2022 ♦ **Accepted** 3 May 2022 ♦ **Published** 3 June 2022

Citation: Belenichev I, Kucherenko L, Pavlov S, Bukhtiyarova N, Popazova O, Derevianko N, Nimenko G (2022) Therapy of post-COVID-19 syndrome: improving the efficiency and safety of basic metabolic drug treatment with tiazotic acid (thiotriazoline). Pharmacia 69(2): 509–516. https://doi.org/10.3897/pharmacia.69.e82596

Abstract

COVID-19 leads to disruption of the blood coagulation system, to thrombosis, hypercoagulability, as a result, to an increased risk of strokes and heart attacks. During COVID-19, endothelial dysfunction develops associated with NO deficiency with decrease in the level of SH compounds. Tiazotic acid (Thiotriazoline) has immunomodulatory, anti-inflammatory, antioxidant, anti-ischemic, cardio- and endothelioprotective, antiplatelet, hepatoprotective activity. Our studies conducted at the National Research Medical Center "University Clinic of ZSMU" with the participation of 57 patients (from 30 to 65 years old) with post-COVID syndrome, who received thiotriazol with basic therapy in either tablets (200 mg each) or suppositories Dalmaxin (0.2 g each) twice a day for 30 days. Inclusion criteria for the study were a positive PCR test for COVID-19; if the PCR test was negative, then the presence of IgM COVID-19 or IgG COVID-19 (with radiologically confirmed pneumonia). The following biochemical parameters were studied: C-reactive protein - by immunoturbodimetric method; D-dimer - by enzyme immunoassay; ferritin - by immunochemiluminescent method; endothelial NO-synthase (eNOS) - by ELISA method; alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyltransferase (GGT), total bilirubin; international normalized ratio (INR) and determination of platelet aggregation. During treatment with thiotriazoline, significant increase in the eNOS content was recorded, which indicated the presence of endothelioprotective activity of the drug. Thiotriazoline significantly reduced the level of D-dimer in the blood of patients, and also led to the normalization of INR. The established effects testified to the presence of antiplatelet and fibrinolytic action of thiotriazoline and its ability to reduce the risks of heart attacks and strokes in post-COVID syndrome. Thiotriazoline led to an objective improvement in general clinical parameters in patients with post-COVID syndrome, complaints of palpitations disappeared, blood pressure stabilized.

Keywords

Tiazotic acid (Thiotriazoline), post-COVID syndrome, antiplatelet, anticoagulant, endothelioprotective and hepatoprotective action

Introduction

Relevance. Coronavirus disease is associated with severe inflammation and cytokine storms (Zhao et al. 2019; Chary et al. 2020; Fan et al. 2020; Wilson et al. 2020). Recently, scientists have been paying more and more attention to the role of autoimmune mechanisms in the pathogenesis of COVID-19, especially when studying the mechanisms of development of complications of this pathology, the most dangerous of which is acute respiratory distress syndrome, which develops in 15–33% of patients (Barnes et al. 2020; Conti et al. 2020; Smeitink et al. 2020). It is believed that one of the main links of its pathogenesis is a cascade of cytokine reactions (hypercytokinemia - IL1β, IL-2, IL-6, IL-7, IL-8, IL-17 IFNγ, G-CSF, MCP1, TNFα, etc.), which is conventionally called "cytokine storm" and occurs in the patient's body as a result of excessive activity of neutrophils and their ability to form extracellular neutrophil traps (NETs). This leads to a logical question about the role of eicosanoids in the pathogenesis of COVID-19, which act as mediators of the inflammatory response and are inextricably linked with signaling cascades realized by cytokines and other signaling molecules (Zhao et al. 2019; Green 2020; Guan et al. 2020; Landi et al. 2020; Varga et al. 2020; Fratta et al. 2021; Lapenna 2021). It is assumed that eicosanoids, especially prostaglandin E2, fulfill one of the leading functions in the development of autoimmune and inflammatory-destructive processes in COVID-19 (Barnes et al. 2020; Chernyak et al. 2020; Conti et al. 2020; Hati and Bhattacharyya 2020; Smith and Smith 2020; Velavan and Meyer 2020; Miller et al. 2021). Inflammation during viral infection leads to oxidative stress, secondary mitochondrial dysfunction, energy deficiency and lactic acidosis in the cell. This leads to damage to cell membranes and cell organelles with reactive oxygen species (ROS), free radicals and peroxidation products, which, in turn, leads to dysfunction and cell death by the type of apoptosis or even necrosis (Conti et al. 2020; Guan et al. 2020; Suhail et al. 2020; Wu et al. 2020; Iqubal et al. 2021; Lapenna 2021; Tyagi and Singh 2021). All this theoretically justifies the prospects of using the original metabolic drug Tiazotic acid (Thiotriazoline) (morpholinium salt of 3-methyl-1,2,4-triazolyl-5-thioacetic acid), developed at RPA «Pharmatron» in 1982, in the complex therapy of COVID-19. Tiazotic acid (Thiotriazoline) has immunomodulatory, anti-inflammatory, antioxidant, anti-ischemic, cardioprotective and hepatoprotective activity. The efficacy of thiotriazoline for these types of activity has been proven both at the preclinical and clinical stages of the study and has been confirmed by more than 20 years of history of use in healthcare in the post-Soviet countries. The main pharmacological effect of thiotriazoline is antioxidant. Thiotriazoline reactivates antioxidant enzymes – glutathioperoxidase (GPx) and superoxide dismutase (SOD), the latter is involved in the protection of proteins from oxidative modification. Thiotriazoline increases the level of reduced glutathione, which regulates the Red/Oxi mechanisms of expression of genes responsible for the synthesis of enzymes, including those that regulate pro-inflammatory cascades – lipoxygenase and cyclooxygenase. Thiotriazoline can directly participate in the regulation of transcriptional activity, prevents the development of imbalance in the thiosulfide system during hyperproduction of ROS, providing such functions as transmission of the cellular signal through the receptor-ionophore complex, preserving the activity of proteins, enzymes, transcription factors and cell integrity (Belenichev et al. 2007, 2019, 2020). There is evidence that thiotriazoline exhibits immunomodulatory activity, increasing the level of interferon, as well as increasing the number of T-lymphocytes. Numerous studies have established that thiotriazoline exhibits anti-inflammatory activity, preventing the irreversible inactivation of the transcription factor NF-Kappa B, and inhibits the expression of pro-inflammatory cytokines – IL-1b, IL-6, TNF-a, as well as C-reactive protein, inducible nitric monoxide synthase – iNOS (Belenichev et al. 2007, 2008; Mazur et al. 2007, 2011). Thiotriazoline stabilizes the basophil membranes of mast cells and eosinophils, increases the phagocytic activity of macrophages.

The very interesting effects of thiotriazoline include its protective effect on the vascular endothelium, which is of great importance in COVID-19, since endothelial dysfunction inevitably develops in this pathology. It is noted that the formation of endothelial dysfunction in COVID-19 occurs more rapidly in elderly patients taking ACE inhibitors (Belenichev et al. 2008, 2019). Endothelial dysfunction is a predictor of such formidable diseases as strokes and myocardial infarctions. It is generally known that NO is an unstable, short-lived radical, and mechanisms such as the formation of stable S-nitrosole complexes with low molecular weight thiol compounds (glutathione, cysteine, methionine) are envisaged for its stabilization and subsequent transportation. Under the conditions of a deficiency of thiol compounds in COVID-19, NO transport is disrupted, since it is attacked by ROS such as superoxide radical and hydroxyl radical with the transformation into a cytotoxic product – peroxynitrite. In this case, there is an increase in the formation of endothelial dysfunction. Reports on preclinical studies of thiotriazoline and dissertation studies have shown that thiotriazoline increases the bioavailability of NO by increasing the level of SH-compounds, as well as independently forming nitrosothiol compounds with NO. All this protects NO from interactions with reactive oxygen species and its transformation into cytotoxic and pro-inflammatory peroxynitrite. Thiotriazoline increases the density of endothelial cells, the density of proliferating endothelial cells, increases the expression of vasculoendothelial factor (VEGF) and endothelial nitric oxide synthase (eNOS) (Belenichev et al. 2008). Clinical studies have shown that the combination of thiotriazoline and arginine leads to a significant increase in the endothelioprotective effect and has a protective effect on the synthesis and transport of NO, its bioavailability. It is known that COVID-19 leads to complications and disrupts blood clotting and thrombus formation. Thiotriazoline has fibrinolytic and antiplatelet properties. Numerous data have been obtained that, in myocardial ischemia, thiotriazoline in platelets significantly increases the activity of glutathione peroxidase, reduces the accumulation of lipid oxidative modification products, which probably leads to a decrease in the blood level of thromboxanes involved in thrombosis (Mazur et al. 2007, 2011; Belenichev et al. 2008). The effect of thiotriazoline on ROSdependent mechanisms of tissue plasminogen expression cannot be ruled out. We have obtained preliminary encouraging results on the positive effect of Tiazotic acid in COVID-19 (Kryvenko et al. 2021). Given the cardiovascular complications caused by both the coronavirus itself and the drugs used in the treatment of COVID-19, data on the cardioprotective effect of thiotriazoline obtained in a number of preclinical and clinical studies (Mazur et al. 2007; Belenichev et al. 2019, 2020). Thiotriazoline reduces mortality, improves ECG, reduces the area of necrosis in experimental myocardial infarction. Thiotriazoline enhances ATP synthesis, normalizes the respiratory chain of mitochondria and increases the utilization of glucose, free fatty acids, glycogen in cells, limits unproductive glycolysis and prevents the development of lactic acidosis in cardiomyocytes, normalizes the work of enzymes in the cycle (more productive and safer than glycolysis) (Mazur et al. 2007; Belenichev et al. 2008). By the strength of the cardioprotective action, thiotriazoline surpasses such well-known cardioprotectors as meldonium, L-carnitine, trimetazidine, inosinium, succinic acid, coenzyme Q10, adenosine 5'-triphosphate. In clinical studies on more than 1000 patients (including senile patients), a positive effect of thiotriazoline on the state of cardiohemodynamics in IHD was shown (Mazur et al. 2007). Thiotriazoline significantly reduced the total peripheral vascular resistance, significantly increased the volume of cardiac output with a progressive decrease in myocardial energy consumption. Along with this, in the group of patients treated with thiotriazoline, exercise tolerance increased, which was accompanied by a noticeable increase in the value of myocardial inotropic reserve (Mazur et al. 2007, 2011; Belenichev et al. 2020). Also, thiotriazoline increased the effectiveness of basic antihypertensive and antianginal therapy. During the appointment of thiotriazoline in patients with acute coronary syndrome, there was a significant decrease in mortality associated with a decrease in the number of ventricular arrhythmias, a more rapid recovery of myocardial function. Good tolerability and safety of course use (8 weeks) of thiotriazoline at a daily dose of 600 mg for the treatment of coronary artery disease, stable angina II-III FC was shown (Belenichev et al. 2008). It has also been established by clinical studies that thiotriazoline reduces the cardiotoxicity of doxorubicin and other anticancer drugs (ECG and biochemistry). Recent data also point to the neurotoxic effect of SARS-CoV-2, in particular, it manifests itself in the form of acute respiratory distress syndrome due to toxic damage to the brainstem, which leads to a disorder of the cardiorespiratory center and respiratory arrest. Preclinical studies have established the neuroprotective activity of thiotriazoline in acute cerebrovascular accident, and clinical - efficiency in the treatment of vascular pathology of the eye (Mazur et al. 2007; Belenichev et al. 2008).

Drug therapy for COVID-19 is aggressive, has a number of serious adverse reactions from the liver and a number of contraindications (patients with liver failure who have had hepatitis, elderly patients). In parallel with the use of the drug in cardiology, tiazotic acid is used in the treatment of diseases of the liver and other internal organs, given the high hepatoprotective properties. The drug prevents the destruction of hepatocytes, reduces the degree of fatty infiltration and the spread of centrilobular necrosis of the liver, promotes reparative regeneration of hepatocytes, normalizes their protein, carbohydrate, lipid and pigment metabolism. Increases the rate of synthesis and excretion of bile, normalizes its chemical composition (Mazur et al. 2007; Belenichev et al. 2019).

The above-mentioned hepatoprotective properties of thiotriazoline can be an essential component of the complex therapy of post-COVID syndrome, given the pronounced hepatotoxicity of drugs used for the basic therapy of coronavirus infection.

Thus, thiotriazoline is a drug with immunomodulatory, antiinflammatory, antioxidant, cardioprotective and hepatoprotective properties; with extensive experience in clinical practice; the safety profile has been carefully studied, which is the basis for its use for the treatment (as part of combination therapy) of patients with post-COVID syndrome. The above is the basis for conducting clinical trials of thiotriazoline for the purpose of its use in the complex therapy of post-COVID syndrome.

The aim of the study. Evaluation of the complex therapeutic effect of thiotriazoline (anticoagulant, antiplatelet, endothelioprotective, hepatoprotective action) in patients with post-COVID syndrome in comparison with basic therapy.

Materials and methods

The studies were carried out on the basis of the ZSMU University Clinic. The studies involved 15 relatively healthy volunteers and 57 patients aged 30 to 65 with post-COVID syndrome. Of these, 20 patients received basic therapy (antibiotics, anticoagulants, acetylsalicylic acid), and 37 patients additionally received thiotriazoline during basic therapy (28 patients received thiotriazoline in the form of tablets (Corporation Arterium, Ukraine (200 mg each), 9 patients - in the form of suppositories Dalmaxin (MobilMedical, Ukraine) 0.2 g each (active ingredient - thiotriazoline) twice a day for 30 days. The inclusion criteria for the study were a positive PCR test for COVID-19; if the PCR test was negative, then the presence of IgM COVID-19 or IgG COVID-19 (with radiologically confirmed pneumonia). The presence of pneumonia was confirmed by computer or X-ray examination of the chest cavity. The level of lung damage is up to 45%. Patients had the following comorbidities: diabetes mellitus in the compensation stage, arterial hypertension, coronary heart disease without heart failure. The following biochemical parameters were studied: C-reactive protein - immunoturbodimetric method (Cormay kit, biochemical analyzer ACCENT-200, Poland); D-dimer - enzyme immunoassay (kit manufactured by "Vector-Best", enzyme immunoassay analyzer - "Immunochem2200", USA); ferritin - immunochemiluminescent method (kit manufactured by Siemens, analyzer - Immulate 1000, UK); endothelial NO synthase (eNOS) – enzyme immunoassay, kit manufactured by Cloud-Clone Corporation, USA (enzymatic immunoassay analyzer – Immunochem-2200, USA). To establish the hepatoprotective effect of thiotriazoline in post-COVID syndrome, a biochemical determination of hepatic enzymes was carried out: alanine aminotransferase (AlAT), aspartate aminotransferase (AST), γ-glutamyltransferase (GGT), the level of total bilirubin (diagnostic kits; a thymol test was performed by the turbodynamic method (RPA "Filisit-Diagnostics"). Also, the international normalized ratio (INR) was determined - the coagulometric method (set manufactured by Diagon, Austria, device - coagulometer CoagChrom 3003, Poland). Simultaneously with biochemical studies, platelet aggregation is determined to assess the hemostatic function of platelets. Platelet aggregation activity was studied by the turbidimetric method (optical aggregometry) using a Solar AP 2110 aggregometer (Republic of Belarus).

Investigation of the level of platelet aggregation activity - with the introduction of the inducer of ADP aggregation (5.0 μM). Material for research: platelet-rich citrate plasma. Two weeks before the study, they stopped taking drugs that affect platelet aggregation. Whole blood was collected in a plastic tube with 3.2% (0.109 M) or 3.8% (0.129 M) sodium citrate in a ratio of 9:1 or in a vacuum blood collection system with 3.2% (0.109 M) sodium citrate. Immediately after blood sampling, the contents of the tube were gently mixed by inverting at least 5 times without foaming. Within 45 minutes, the tube was delivered to the laboratory and centrifuged. Whole blood sample centrifugation was performed at room temperature (18–25 °C) for 5–7 minutes at 1000 rpm. After centrifugation was completed, 1 ml of TRP was immediately taken into a clean tube for further study. Obtaining platelet-poor plasma (PPP) is used as a blank sample (reference point). To obtain platelet-poor plasma, a whole blood sample was centrifuged at room temperature (18–25 °C) for 15 minutes at 3000 rpm. After centrifugation was completed, 1 ml of PRP was taken into a clean plastic tube. Blood sampling was performed only in vacuum systems or plastic tubes with 3.8% sodium citrate. Before the analysis, a preliminary count of cells in plasma was carried out on a hematological analyzer or a microscopic method, and in accordance with the results obtained, platelet-rich plasma was diluted with platelet-poor plasma (from the same patient) so that the total number of platelets in the mixture was 200–300×109/l. An ADP solution with a concentration of 5.0 μM was used as an aggregation activator. To prepare a working solution, 4.7 mg of ADP was added to 20 ml of saline, then 1 ml of the resulting solution was added to 9 ml of saline. The results obtained were measured by the percentage of light absorption. The results of the study were calculated using the standard statistical package "STATISTICA for Windows 6.0" (StatSoftInc., No. AXXR712D833214FAN5), as well as "SPSS 16.0", "Microsoft Office Excell 2003". Distribution normality was assessed using the Shapiro-Wilk test. Data were presented by mean. The significance of negativity between the mean values was determined by Student's t-test (in the case of a normal distribution). In the case of a distribution that is negative from normal, or analysis of ordinal variables, the U Mann-Whitney test was used. To compare independent variables in more than two samples, analysis of variance (ANOVA) with a normal distribution or the Kruskal-Wallis test for a distribution that differed from normal in the negative direction was used. For all analyzes, negatives $p<0.05$ (95%) were considered statistically significant.

Results and discussion

Upon admission, all patients complained of severe weakness, increased fatigue, palpitations, fever from 37.2 to 38.3 °C. The level of lung damage is up to 45%. Complaints about the lack of smell and taste had 51% of patients, cough - 49.1%, shortness of breath - 43%, diarrhea and abdominal pain - an average of 24.5% (Table 1). Patients also noted fluctuations in blood pressure, especially those with concomitant arterial hypertension. Fluctuations were noted despite the constant use of specific therapy (calcium channel blockers, ACE inhibitors, sartans, beta-blockers). After treatment, in the group of patients who took thiotriazoline, complaints of palpitations disappeared, blood pressure stabilized (without additional correction with antihypertensive drugs), weakness and fatigue disappeared. Saturation in 35 (94.6%) patients of the main group increased to 98–99%. In the control group, 9 (45%) of 20 patients had a saturation of 98% (Table 1).

Table 1. Subjective state of patients at admission and 1 month after treatment.

Complaints/indicators	On admission n=57	Group 1 - basic therapy (control) after treatment n=20	Group 2 - basic therapy + thiotriazoline (after treatment) (n=37; 28 patients received tablets, 9 received suppositories)
Weakness	55	17	2
Body temperature from 37.2° to 38.3°	55	–	–
No sense of smell or taste	29	7	3
Dyspnea	25	11	2
Cough	28	8	–
Heartbeat	55	16	3
Rhythm disturbance	–	–	–
Diarrhea	14	2	–
Abdominal pain	14	2	–
Fatigue	54	14	2
Saturation at the level of 98–99%	–	9	35

The conducted biochemical and coagulometric studies showed that in patients with post-COVID syndrome, eNOS expression derivation was established with an increase in the concentration of ferritin and C-reactive protein in relation to relatively healthy ones. Studies have shown that in patients with post-COVID syndrome during treatment with basic therapy (antibiotics, anticoagulants, acetylsalicylic acid), compared with relatively healthy patients, an increased concentration of C-reactive protein and ferritin was observed (Table 2) during INR with a reduced plasma eNOS concentration blood. However, in this group, a significant decrease in C-reactive protein was observed compared with the data before the start of treatment (Table 3). When examining the content of D-dimer, no statistically

Table 2. Biochemical parameters of blood plasma and INR in patients with post-COVID syndrome (30 days from the start of treatment).

Groups of patients	C-reactive protein, mg / l	Feritin, ng / ml	D-dimer DDU	INR
Relatively healthy (п=15)	9,1±0,8	330±10,1	130,2±14,6	0,92±0,04
On admission n=57	21,4±2,8	478,3±7,6	190,3±6,1	0,44±0,03
Post-COVID syndrome + basic therapy (n=20)	20,4±1,2[1]	409,2±6,5	155±10,2	0,58±0,05
Post-COVID syndrome + basic therapy + thiotriazoline (n=37; 28 patients received tablets, 9 received suppositories)	19,9±1,3[1]	388±7,3*[1]	132±6,5*[1]	0,89±0,03*[1]

[1]- p<0,05 in relation to patients on admission;
* - p<0,05 in relation to patients with post-COVID syndrome and basic therapy.

possible changes were found (reference values up to 285 DDU). The introduction of thiotriazoline into the basic therapy (within 1 month) (Table 2) led to the normalization of INR (a significant increase in relation to the group of patients when staying by 50% and in relation to the group of basic therapy - by 35%) and an increase in the content of eNOS (a significant increase in relation to the group of patients at admission by an average of 55% and in relation to the group of basic therapy - by 42%), a decrease in D-dimer (a significant decrease in relation to the group of patients at admission by 31% and in relation to the group of basic therapy - by 15%). INR (International Normalized Ratio) is one of the studies for prothrombin. With its help, the state of the blood coagulation system in the patient is determined. This protein is a precursor of thrombin, a protein that stimulates the formation of a blood clot. A decrease in eNOS is a sign of endothelial dysfunction. D-dimer is a more specific marker of the degradation of fibrin clots of any localization, in other words, a marker of the intensity and nature of the action of thrombus formation. An increase in the concentration of D-dimer clearly and unambiguously indicates the activation of fibrinolysis, which, in turn, is necessarily preceded by excessive formation of insoluble fibrin, that is, thrombus. The introduction of thiotriazoline into the basic therapy (Table 2) led to a decrease in ferritin (a significant decrease in relation to the group of patients in the presence of 19% and in relation to the group of basic therapy - by 5.1%). Indicators of C reactive protein did not statistically differ from similar values in the control group of patients.

When determining the aggregation activity of platelets, it was found that in patients with post-COVID syndrome during treatment with basic therapy, compared with healthy patients, an increase in platelet aggregation activity was observed. The percentage of light absorption

Table 3. Concentration of eNOS in the blood plasma of patients with post-COVID syndrome (30 days from the start of treatment).

Groups of patients	eNOS, pg / ml
Relatively healthy (n=15)	57,8±4,3
On admission (n=57)	24,1±5,2
Post-COVID syndrome + basic therapy (n=20)	31,4±4,7
Post-COVID syndrome + basic therapy + thiotriazoline (n=37; 28 patients received tablets, 9 received suppositories)	54,1±4,7*[1]

[1]- p<0,05 in relation to patients on admission
* - p<0,05 in relation to patients with post-COVID syndrome and basic therapy.

Table 4. Hemostasiogram of patients with post-COVID syndrome (30 days from the start of treatment) (%).

Groups of patients	Platelet aggregation with ADP, %	Speed at 30 seconds, %	Number of platelets, 10^9/l
Relatively healthy (п=15)	60±10,4	70±15,2	311±31,4
Post-COVID syndrome + basic therapy (п=20)	100,4±7,3	161,4±8,6	380±53,6
Post-COVID syndrome + basic therapy + thiotriazoline (n=37; 28 patients received tablets, 9 received suppositories)	68,5±4,8*	74,6±7*	338±26,1

*- p<0,05 in relation to patients with post-COVID syndrome and basic therapy.

averaged 99.4% versus 60% of relatively healthy patients (Table 4; Fig. 1A, B). at the same time, there was an increase in speed for 30 seconds while maintaining a normal platelet count (380 10^9/ l ±54,8). The indicators of relatively healthy patients did not differ from the reference values (platelet aggregation – 50–80%; speed at 30 seconds – 58–114%; platelet count – 260–600 10^9/l). The introduction of thiotriazoline into basic therapy (within 1 month) (Table 3, Fig. 1C) led to a decrease in platelet aggregation activity by 32% and aggregation rate at 30 seconds – 54%. It should be noted that the indicators of patients in this group, as can be seen from Table 4 decreased to levels of relatively healthy patients.

We have obtained data on the protective effect of thiotriazoline on the vascular endothelium, which is of great importance in COVID-19, since endothelial dysfunction inevitably develops in this pathology. It has been noted that the formation of endothelial dysfunction in COVID-19 occurs more rapidly in elderly patients taking ACE inhibitors. Endothelial dysfunction is a predictor of such formidable diseases as strokes and myocardial infarctions. It is well known that NO is an unstable, short-lived radical, and for its stabilization and subsequent transportation, mechanisms such as interaction with thiol-containing low molecular weight compounds (glutathione, cysteine, methionine) and reproduction of stable S-nitrosol complexes are provided. Under conditions of deficiency of thiol compounds in COVID-19, NO transport is disrupted, as it is attacked by such ROS as superoxide radical and hydroxyl radical with transformation into a cytotoxic product, peroxynitrite (Belenichev et al. 2008, 2020b, Mazur et al.2007). At the same time, there is an increase in the formation of endothelial dysfunction. Preclinical reports of thiotriazoline and dissertation studies have shown that thiotriazoline increases NO bioavailability by increasing the level of SH compounds, as well as self-forming nitrosothiol complexes with NO. All this protects NO from interactions with reactive oxygen species and its conversion into cytotoxic and pro-inflammatory peroxynitrite. Thiotriazoline increases the density of endotheliocytes, the density of proliferating endotheliocytes, increases the expression of vascular endothelial growth factor (VEGF) and endothelial nitric monoxide synthase (eNOS). Clinical studies have shown that the combination of thiotriazoline and arginine leads to a significant increase in the endothelial protective effect and has a protective effect on the synthesis and transport of NO, its bioavailability (Belenichev et al. 2007, 2008, 2020b,

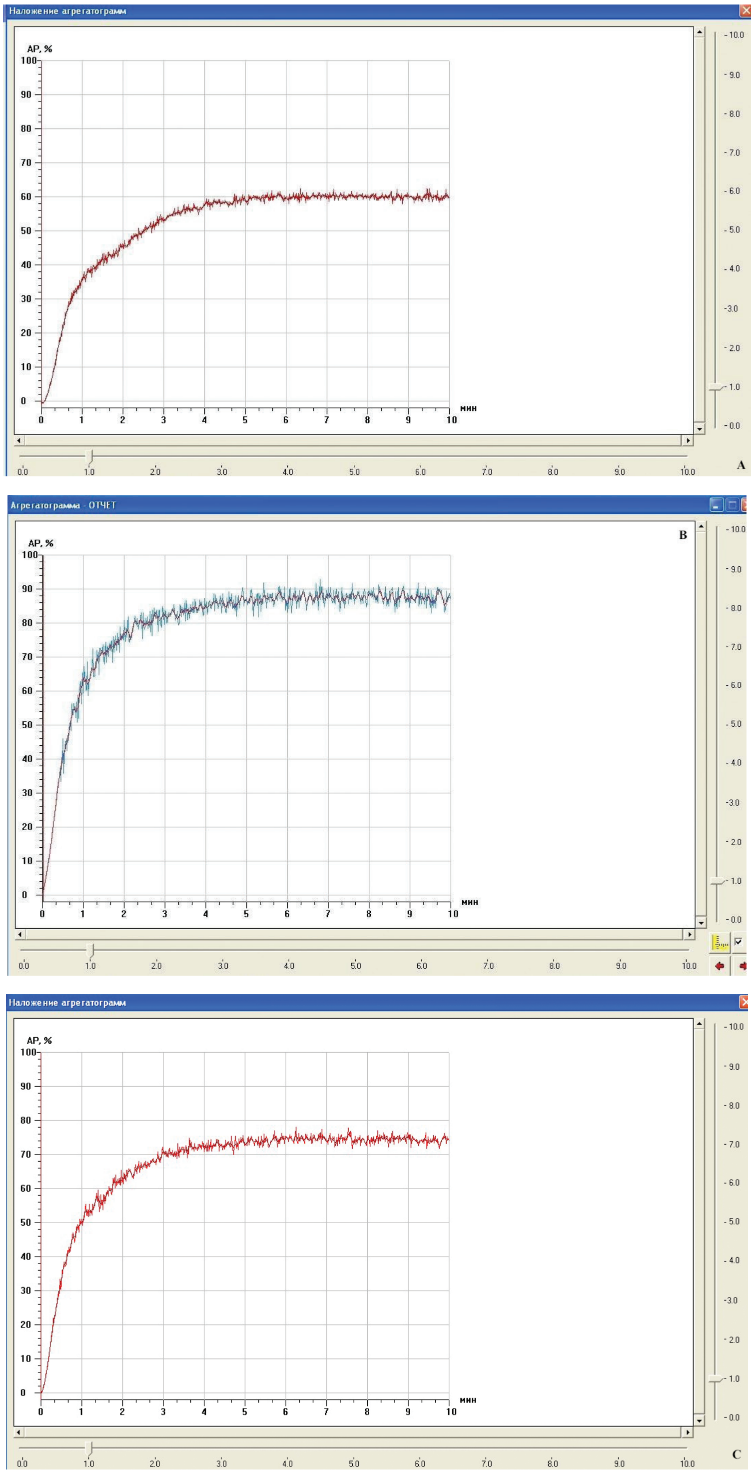

Figure 1. Platelet aggregation activity. **A.** Relatively healthy patients. Aggregation response in the reference interval. Irreversible aggregation, percentage light transmission 60%; **B.** patients with post-COVID syndrome during treatment with basic therapy. Aggregation response in the reference interval. Irreversible aggregation, percentage light transmission 93%; **C.** Patients with post-COVID syndrome during treatment with basic therapy and thiotriazoline. Aggregation response in the reference interval. Irreversible aggregation, percentage light transmission 75%.

Mazur et al. 2007). Data were also obtained on the anticoagulant effect of thiotriazoline. It is known that COVID-19 leads to complications and disrupts blood clotting and thrombosis. Thiotriazoline exhibits anticoagulant and antiplatelet properties. This Numerous data have been obtained that, in myocardial ischemia, thiotriazoline in platelets significantly increases the activity of glutathione peroxidase, reduces the accumulation of lipid oxidative modification products, which probably leads to a decrease in the blood level of thromboxanes involved in thrombosis. The effect of thiotriazoline on ROS-dependent mechanisms of tissue plasminogen expression cannot be ruled out.

One of the important components of the post-COVID syndrome is the development of adverse side effects of drugs used to treat coronavirus disease, namely, violations of the hepatobiliary system and the protective function of the liver. In patients, an increase in liver enzymes in the blood plasma, an increase in the content of bilirubin was observed. In 54 out of 57 patients during hospitalization, asthenovegetative, cholestatic, pain syndrome in the epigastrium and right hypochondrium was recorded.

The introduction of thiotriazoline into basic therapy in the form of tablets (200 mg twice a day) or suppositories (200 mg twice a day) for 30 days led to a decrease in the manifestation of the cytolytic syndrome, which was manifested in a decrease in ALT and AST by 55.7%. and 66.7% compared with the group of patients at admission and 48.7% and 60.6%, respectively, compared with the group of patients receiving basic therapy. It is important to note that in the group of patients who received basic therapy, the AST value remained elevated (Table 5). In addition, the protein-synthesizing function of the liver was normalized, as evidenced by the normalization of the thymol test, in contrast to the group of patients who received basic therapy; there was also a decrease in total plasma bilirubin and GGT levels (Table 5). In 35 patients out of 37 (94.6%) who received a course of thiotriazoline, there was a positive trend, and in itself a change in asthenovegetative, cholestatic, pain syndrome in the epigastrium and right hypochondrium. In the group receiving basic therapy, positive dynamics was observed only in 9 patients out of 20 (45%).

According to previous studies, the mechanism of the hepatoprotective action of thiotriazoline lies in its antioxidant, membrane-protective and mitoprotective activity. Thiotriazoline is able to protect the enzymes of the pentose phosphate shunt, the tricarboxylic acid cycle in hepatocytes from their oxidative damage, which ensures a sufficiently high level of energy and plastic processes in the liver tissue (Belenichev et al. 2007, 2019). In addition, due to the presence of free SH-groups in the molecular structure of thiotriazoline, it is able to bind and inactivate cytotoxic derivatives of oxidative stress and xenobiotic metabolites (Mazur et al. 2007, Belenichev et al. 2020a, c). Thiotriazoline protects the membranes of the liver mitochondria in toxic hepatitis. This effect is confirmed by the preservation of the mitochondrial membrane potential and the functional preservation of the cyclosporin A-dependent mitochondrial pore. The membrane-stabilizing activity of thiotriazoline is realized by inhibiting the processes of lipid peroxidation in the membranes of the endoplasmic reticulum of the liver (decrease in the formation of MDA, inhibition of biochemiluminescence) in case of toxic liver damage. The membrane-protective activity of thiotriazoline is also manifested in the ability to normalize a number of physicochemical parameters of the membrane structure (fluorescence of 1,8-anilino-8-ammonium sulfonate, demonstrating the integrity of membranes, intrinsic protein fluorescence, Stern-Volmer constant (free radical quenching rate), microviscosity.

Table 5. Biochemical parameters of the liver tissue of patients with post-COVID syndrome (30 days from the start of treatment).

Groups of patients	ALAT U / l	ACT, U / l	GGT, U / l	Thymol test, Sh	Total bilirubin, μmol / l
Relatively healthy (п=15)	18,4±,1,5	9,2±0,6	24,5±2,5	1,2±0,07	12,8±1,5
On admission (n=57)	64,2±3,8	52,1±2,7	82,4±3,4	11,4±2,2	22,7±1,8
Post-COVID syndrome + basic therapy (n=20)	55,4±3,7*	44,2±1,1*	78,4±2,3	6,7±0,5*	20,4±1,8
Post-COVID syndrome + basic therapy + thiotriazoline (n=37; 28 patients received tablets, 9 received suppositories)	28,4±2,8*[1]	17,4±1,2*[1]	26,4±1,7[1]	0,7±0,05*[1]	11,3±0,9*[1]

[1]- p<0,05 in relation to patients on admission;
* - p<0,05 in relation to patients with post-COVID syndrome and basic therap.

Conclusion

The obtained clinical and biochemical results demonstrate the hepatoprotective effect of thiotriazoline. And given a number of serious side effects of basic drugs (antibiotics, antiviral agents, NSAID antiplatelet agents) aimed at disrupting the subtle links of the metabolism of cardiomyocytes, endotheliocytes, hepatocytes, etc., the appointment of thiotriazoline in the complex therapy of post-COVID syndrome can increase the safety of the proposed drug treatment.

Thus, the introduction of the drug tiazotic acid (thiotriazoline) in the form of tablets (200 mg twice a day) or suppositories Dalmaxin (200 mg twice a day) into the complex basic therapy of the post-COVID syndrome for 30 days led to a significant increase in the basic endothelioprotective anticoagulant therapy, contributed to the prevention of thrombus formation while improving the condition of the myocardium and vascular endothelium, and also reduced disorders of the hepatobiliary system caused by both the disease itself and side effects of basic therapy.

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