护理杂志
Journal of Nursing
Volume 72 Number 02
ISSN: 0047-262X
DOI: 10.5281/zenodo.14864969

Study of Antioxidant Properties and Influence On Mitochondrial Bioenergetics of a Composition Consisting of Mono Ammonium Salt of Glycyrrhizic Acid and Amino Acids

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Abstract

The antioxidant effect and influence of a composition consisting of monoammonium salt of glycyrrhizic acid (MASGA) and amino acids - cysteine, methionine, and tryptophan on the bioenergetics of rat liver mitochondria in paracetamol-induced liver damage in rats were studied. It has been established that paracetamol causes a significant increase in the liver homogenate of diene conjugates and conjugated trienes, as well as a secondary product of lipid peroxidation (LPO) - malondialdehyde (MDA). In the liver mitochondria, under the influence of paracetamol, there is a decrease in respiration and oxidative phosphorylation processes. The introduction of the studied composition to hepatitis animals reduced the content of primary and secondary LPO products and partially restored the functional state of the mitochondria.

Keywords: *Antioxidant, Mitochondria, Paracetamol, Lipid Peroxidation, Malondialdehyde, Cytochrome P450, Amino Acids.*

INTRODUCTION

It is known that energy metabolism disorders trigger the development of pathological processes in the cell [1,2]. Many drugs in therapeutic doses affect the energy production system in the liver. In the case of liver poisoning with paracetamol, the following sequence of events occurs: initial cell damage, change in the permeability of mitochondrial membranes, and subsequent cell death. In this case, inhibition of enzymes, blocking or shunting of the mitochondrial respiratory chain, and uncoupling of the oxidative phosphorylation process are observed [3]. Under the influence of paracetamol, the latter's free radicals and free calcium ions reduce the transmembrane potential mitochondria, causing the formation of pores and stimulation of the synthesis of proinflammatory cytokines [4]. Apoptosis-inducing factors exit the mitochondria through pores, causing disturbances in the nuclear and mitochondrial DNA of hepatocytes [5]. As a result, hepatitis and even fulminant liver failure develop [6].

To treat such conditions, substances with antioxidant properties are used, as well as the ability to restore the functional activity of liver cell mitochondria. It is of interest to study the effect of a composition consisting of monoammonium salt of glycyrrhizic acid (MASGA) and amino acids - cysteine, methionine, and tryptophan.

Glycyrrhizic acid (GA) and its derivatives have pronounced antioxidant and anti-inflammatory properties. In a damaged cell, they directly increase the expression of the nuclear factor Nrf2, which

Volume 72 Number 02
ISSN: 0047-262X
DOI: 10.5281/zenodo.14864969

in turn enhances the transcription of antioxidant protein genes [**7**]. In addition to its antioxidant action, glycyrrhizic acid has anti-inflammatory and anti-apoptotic effects by suppressing TNF-α and caspase-3. The amino acids cysteine, methionine, and tryptophan can reduce oxidative stress in the liver and other tissues under the influence of toxic compounds [8].

Purpose of the research

This study aims to investigate the antioxidant properties of the composition MASGA/cysteine, methionine, and tryptophan, and its effect on the functional state of liver mitochondria during experimental damage with paracetamol.

MATERIAL AND METHODS

The study involved white mongrel rats of both sexes weighing 160-180 g. Drug-induced liver damage was reproduced by intragastric administration of paracetamol at a dose of 500 mg/kg for 2 days [9]. All animals were divided into 4 groups. The first group was a control group; the second group consisted of model animals that received paracetamol. The third group consisted of rats that were administered 5 mg/kg of the MASGA/amino acid composition. The fourth group of animals was administered the comparison drug STD. The comparison drug was the injectable drug Stronger Neo- Neo-Minophagen C (Japan) (STG), a compound with high hepatoprotective properties, containing glycyrrhizic acid and the amino acids Glycine and L-Cysteine.

The composition of MASGA/amino acids and the reference drug were administered to hepatitis animals for 12 days. Mitochondria from the liver of experimental and control rats were isolated by the conventional method of differential centrifugation. The respiratory rate and parameters of oxidative phosphorylation were recorded by the polarographic method using a rotating platinum electrode. 1 10-3 M succinate, 3 10-3 M glutamate, and malate were used as oxidation substrates. The mitochondrial respiration rate was recorded before (V_{4n}), during (V_3), and after (V_{4o}) the ADP phosphorylation cycle, added in an amount of 125 μmol. In all measurements, the absolute values of the rates of oxygen consumption by mitochondria are presented as ng of atomic oxygen/min/mg of protein. To assess the energy status, the coupling coefficient of oxidative phosphorylation (ADP/O) was calculated. [10]. In liver homogenate, the activity of lipid peroxidation (LPO) was studied by the rate of formation of spontaneous and ascorbate-dependent malondialdehyde (MDA), the content of diene conjugates (DC) and conjugated trienes (CT) [11]. Protein in the samples was determined by the biuret reaction [12].

RESULTS AND DISCUSSION

Paracetamol (acetaminophen) is a non-steroidal anti-inflammatory drug and is used as an analgesic and antipyretic agent [13]. In therapeutic doses, paracetamol is safe, does not have a damaging effect on the gastrointestinal tract, and does not have adverse cardiorenal effects. Overdose of paracetamol leads to liver necrosis and liver failure, the development of oxidative stress, which is accompanied by an increase in the process of lipid peroxidation, oxidation of DNA and proteins, and a decrease in antioxidant protection [14].

In the first series of studies, the content of primary and secondary products of lipid peroxidation - diene conjugates (DC), conjugated trienes (CT), and MDA in liver homogenate in toxic hepatitis caused by the administration of paracetamol (Table 1) was studied.

护理杂志
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Table 1: Changes in the content of primary and secondary lipid peroxidation products in the liver of rats with paracetamol hepatitis and the introduction of the composition MASGA/amino acids (n=8; M±m)

		Primary products of LPO (nmol / mg lipids)		Secondary products LPO (MDA) nmol / mg protein /min	
		DK	ST	Ascorbate is dependent.	NADH dependent
1	Control	1.09±0.091	0.38±0.023	0.340±0.04	0.365±0.015
2	Paracetamol Hepatitis (PH)	3.38±0.11	1.27±0.11	0.765±0.013	0.891±0.011
3	STD	2.54±051	0.77±0.02	0.526±0.57	0.578±0.023
4	PG+MASGA/amino acids (5mg/kg)	2.85±0.12	0.83±0.01	0.505±0.011	0.529±0.016
5	PG+MASGA/amino acids (10mg/kg)	2.43±0.01	0.71±0.02	0.491±0.017	0.485±0.011

It was found that during paracetamol intoxication in liver homogenates, the formation of NADH-dependent (spontaneous) and ascorbate-dependent MDA accelerated by 2.25-2.44 times. The content of diene conjugates and conjugated trienes increased by 3.2-3.34 times. Correction of the detected disturbances in the content of LPO products by different doses of the composition MASGA/amino acids inhibited the activation of LPO at: when using a dose of MASGA/amino acids of 5 mg/kg, the formation of spontaneous and ascorbate-dependent MDA slowed down by 1.4-1.5 times, the amount of diene conjugates and conjugated trienes decreased by 2.0-2.2 times (Table 1). A twofold increase in the dose of the studied composition led to an even greater decrease in the LPO process in the liver cells of hepatitis animals.

It is known from the literature that in almost all cases paracetamol initiates lipid peroxidation: in the blood serum of rabbits that were administered the drug (1g/kg body weight) for 9 days, an increase in the concentration of malonic acid was observed dialdehyde [15]. In rats, exposure to various doses of paracetamol was also accompanied by an increase in the level of MDA in the blood serum and liver tissue [16]. Moreover, such an increase in intermediate peroxidation products correlated with the level of inhibition of the antioxidant system - a decrease in the level of glutathione and enzymes involved in its metabolism, as well as the level of superoxide dismutase and catalase [17]. Similarly, an increase in lipid peroxidation in the liver or blood serum of animals is observed with long-term administration of lower doses of paracetamol (100 and 250 mg/kg body weight) [18]. Analysis of literary data shows that in the process of paracetamol metabolism with the participation of cytochrome P450, a toxic metabolite N-acetyl-p- benzoquinone imine (N - acetyl -p- benzoquinone imine) is formed. imine, NAPQI), which conjugates with reduced glutathione even at low drug concentrations [19, 20]. When exposed to high doses of paracetamol or under conditions of glutathione depletion, the toxic metabolite NAPQI additionally reacts with cellular proteins, causing oxidative stress, lipid peroxidation, free radical generation, and mitochondrial damage, which leads to necrotic or apoptotic death of hepatocytes [21].

Administration of various doses of the composition MASGA/amino acids to animals poisoned with paracetamol reduced the process of lipid peroxidation in liver cells, and the dose of the composition equal to 10 mg/kg had a more significant effect in all cases of studying the antioxidant action of the drug, even in comparison with the action of the comparison drug - STD. Such a pronounced antioxidant action of the studied composition represents the sum of the effects of MASGA and amino acids.

The main pharmacological action of Glycyrrhizin acid (GA) and its derivative MASGA is anti-inflammatory. It is realized with the participation of a whole complex of mechanisms. GA inhibits the release of TNF-α, the activity of myeloperoxidases, and the translocation of nuclear factor-kB

护理杂志
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(NF-kB) into the nucleus. GA and MASGC also suppress the production of IL-6, IL-1, and inhibit mitogen-activated protein kinases (MAPKs) [22].

In addition, the anti-inflammatory action of these compounds is explained by antioxidant activity - binding of free oxygen radicals; an increase in the content of glutathione in liver cells due to the inhibition of its secretion with bile; suppression of the synthesis of NO and the production of highly reactive forms and compounds of oxygen, such as O_2, H_2O_2, OH [22, 23]. Experimental studies have demonstrated that GA and its derivative glyceram prevented oxidative stress caused by lead acetate by binding this element. GA also reduces inflammation and the progression of liver fibrosis caused by toxins, including alcohol. This was accomplished by inhibiting the proliferation of CD4+T cells in response to toxic effects.

In addition to the ability of GA and its derivatives to inhibit necrosis and apoptosis of hepatocytes due to the suppression of TNF-α and caspase-3, they cause a decrease in the release of cytochrome-C from mitochondria [24]. GA and MASGA also have a stabilizing effect on membrane permeability. The antifibrotic effect of GA is explained by the suppressive effect on collagen production by hepatic stellate cells and the destruction of activated Ito cells with the participation of natural killers [23]. In addition, GA and MASGA have immunomodulatory properties due to the stimulation of endogenous interferon production; activation of natural killers; increased IL-2 production; a decrease in the concentration of immunoglobulin E, IL-4, IL-5, IL-6, nitric oxide, TNF-α, NO synthase activity in the blood; an increase in the content of immunoglobulins A, G, M, and IL-12 in the blood [22].

The amino acids cysteine, methionine, and tryptophan, which are part of the composition under study, also have a variety of biological activities. Methionine and cysteine sulfur-containing amino acids-antioxidants are very sensitive to various free radicals. In addition, methionine and cysteine participate in synthesizing S-adenosylmethionine, hydrogen sulfide, taurine, and glutathione. In various model experiments, the listed compounds reduced oxidative stress to varying degrees and protected various tissues from damage [8, 25].

Tryptophan is also an essential amino acid, is part of various proteins, as well as essential substances such as serotonin, melatonin, tryptamine, niacin, quinolinic acid, nicotinamide adenine dinucleotide [26]. Tryptophan and its metabolites effectively inhibit free radicals, including active forms of oxygen and chlorine. It has been established that tryptophan very actively inhibits the release of proinflammatory cytokines - TNF-α and IL-6-ga - which is an important component of the immune system [27].

Thus, the suppression of the LPO process in the liver of rats poisoned with paracetamol, detected in our experiments, may be the result of the combined effect of MASGA and amino acids - cysteine, methionine and tryptophan. In the next series of studies, we studied the functional state of the liver mitochondria of rats poisoned with paracetamol and the correction of the detected disorders by different doses of the MASGA/amino acid composition. The data are presented in Table 2.

Indeed, paracetamol intoxication also contributed to the development of de-energization in the liver. mitochondria by the kinetic type, which is characterized by a discrepancy between the dynamics of consumption and synthesis of macroergs under conditions of increased load on the energy production system (Table 2). The oxidation rates of endogenous substrates before (V4n), during (V_{3}), and after (V_{4o}) of the ADP phosphorylation cycle became 26-73% higher than normal. In the experiment with oxidation of exogenous succinate, the respiration rate in the $V_{3\ state}$ did not differ significantly from normal, the respiration rates in the V4n and V4o states increased by 19-21%. During the oxidation of NAD-dependent substrates by mitochondria of the liver damaged by

护理杂志
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paracetamol, the respiration rates increased by 15-35%. During the oxidation of all substrates, the ADP/O ratio decreased (by 1-36%).

Table 2: The effect of the composition of MASGC/amino acids on the functional state of Crimean liver mitochondria during experimental paracetamol intoxication (n=10; M±m)

Indicators	Intact animals	Paracetamol	Paracetamol+		
			STD	MASGC/amino acids 5mg/kg	MASGC/amino acids 10mg/kg
Succinate oxidation					
V_{4n}	50.07±1.52	60.09 ±1.11	54.60±1.311	51.21±1.21	48.21 ±1,21
V_3	95.21±1.09	99.72±0.82	102.35±1.23	117.49 ±1.35	86,91 ±1.22
V_{4o}	45.11±0.14	52.23 ±1.03	56.12±1.82	56.09 ±1.11	39.19±1.02
ADP/O	2.08±0.10	1.58±0.05	1.95±0.05	1.97±0.07	1.99±0.02
Oxidation of NAD-dependent substrates (Malate + glutamate)					
V_{4n}	34.01±0.04	45.27±0.43	44.86±0.65	45.99±0.37	368.78±0.17
V_3	74.19±0.55	80.95±0.58	84.89±0.48	86.15±0.33	68.99±0.65
V_{4o}	29.34±0.16	38.96±0.51	43.11±0.42	45.30±0.574	47.03±0.11
ADP/O	2.71±0.02	2.09±0.05	2.59±0.16	2.67±0.08	2.84±0.13

The results obtained in this series of studies are consistent with the results of Prot studies. J. M. and others showed changes in mitochondrial membrane potential and the development of mitochondrial dysfunction in the liver under the influence of high doses of paracetamol [28]. The authors were able to reproduce the pathways of glutathione depletion by forming NAPQI. When conducting experiments using liver cell culture, it was found that the specific increase in the consumption of cysteine, histidine, and methionine in cells cultured on microfluidic chips correlates with the intensity of the glutathione pathway, which is involved in the detoxification of paracetamol. Under metabolic stress, 2-hydroxybutyrate is also released as a by-product of the breakdown of cystathionine to cysteine before its conversion to glutathione [29]. In addition, the glutathione precursor S- S-adenosylmethionine is formed by the conjugation of methionine with ATP and cysteine, which is involved in the synthesis of glutathione. Elevated levels of these molecules in the medium during the cultivation of primary hepatocytes with paracetamol are a marker of toxic cell damage.

Thus, as a result of the conducted studies it was established that paracetamol disrupts the respiratory function of mitochondria against the background of increased need for energy resources. This is accompanied by the activation of controlled respiration and a moderate response to the load after adding ADP. From the mitochondria through membranes damaged by paracetamol into the cytoplasm, first of all, SDH, then I complex of the respiratory chain. Paracetamol has the least toxic effect on the ascorbate-dependent complex of the respiratory chain.

The use of the MASGK/amino acid composition against the background of paracetamol intoxication increased the coupling of oxidation and phosphorylation in rat liver mitochondria (the ADP/O ratio increased by 14-21%), although the kinetic parameters of tissue respiration were almost identical to the values recorded in untreated animals. In rat liver mitochondria, the rate of oxygen uptake in the V4o state increased by 12% ($p < 0.05$) during succinate oxidation. During the oxidation of NAD-dependent substrates, the rate of liver mitochondrial respiration increased in the V4o state by 15% ($p < 0.05$) (Table 2).

When a high dose of the MASGC/amino acid composition was administered against the background of paracetamol intoxication, liver bioenergetics improved even more. Most functional indices of mitochondrial respiration changed significantly towards the norm. Respiration rates during

Volume 72 Number 02
ISSN: 0047-262X
DOI: 10.5281/zenodo.14864969

oxidation of endogenous substrates, exogenous succinate, and NAD-dependent substrates decreased by 12-40%. The ADP/O ratio increased by 15-27%.

Thus, in paracetamol intoxication of rats, a high dose of the MASGC/amino acid composition has a pronounced antioxidant effect and restores the respiratory function of liver mitochondria. Correction of these conditions with 10 mg/kg of the MASGC/amino acid composition reduced the LPO process in the homogenate and increased the coupling of oxidation and phosphorylation in liver mitochondria in paracetamol poisoning.

CONCLUSION

Despite a long history of clinical use and a good study of the therapeutic effects, the mechanism of action of paracetamol remains unclear to this day. At the same time, paracetamol (acetaminophen) is a dose-dependent hepatotoxin and its overdose is the most common cause of drug-induced liver injury in various countries [30]. The liver metabolizes the bulk of therapeutic doses of paracetamol by its glucuronide and sulfate conjugation. Only a small amount is converted to the highly reactive derivative NAPQI to bind via the cytochrome P450-dependent oxidase system. The ability of NAPQI to bind sulfhydryl groups of mitochondrial proteins hepatocytes leads to inhibition of mitochondrial respiration, increased oxidative stress, and the development of mitochondrial dysfunction with depletion of ATP reserves [30, 31]. The biochemical processes described above lead to a change in homeostasis, and an increase in the permeability of the cell membrane with subsequent cellular swelling, vacuolization, and loss of cellular elements, representing one of the biochemical signs of hepatocyte necrosis [24, 25].

To correct such disorders, substances with antioxidant activity and the ability to restore the functional state of mitochondria are used. The high dose of the MASGK/amino acid composition studied in our experiments has a pronounced antioxidant effect and restores the respiratory and ATP-forming function of liver mitochondria.

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