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DYNAMICS OF NON-HEME IRON CONTENT IN MYOCARDIUM AND HEME OXYGENASE ACTIVITY UNDER HYPERERGIC CONDITIONS

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Iron is the primary element involved in gas transport by blood and is a component of antioxidant enzymes within cells. Free iron, which is not bound to proteins or other molecules, can be chemically active and play different roles in cells and tissues. However, its high chemical activity can also lead to the formation of free radicals, which can damage cells and cause oxidative stress.
In heart metabolism, where free radicals and oxidative stress can be harmful, control of free iron is very important. The body uses various mechanisms to control free iron levels, such as iron-binding proteins and antioxidants, to ensure the right balance and prevent possible damage.
The dynamics of non-heme iron content in the myocardium were investigated under conditions of "adrenaline shock". The amount of non-heme iron in the myocardium and blood plasma was determined at the beginning, middle, and end of the experiment. Simultaneously, the content of malonic dialdehyde, diene conjugates, superoxide dismutase, catalase, reduced glutathione, and hem oxygenase activity was also determined.
By the end of the experiment, the content of non-heme iron in the myocardium had decreased, coinciding with an increase in the activity of hemoxygenase-1. Conversely, plasma levels of non-heme iron had increased, along with an elevation in oxidative stress markers such as malondialdehyde and diene conjugates in blood plasma. The antioxidant enzyme superoxide dismutase showed a decrease initially, followed by restoration towards the end of the experiment. A similar pattern was observed in the level of reduced glutathione. These findings suggest that under conditions of myocardial metabolic damage, hem oxygenase protection is activated to counteract excessive non-heme iron accumulation, thereby shielding the heart from oxidative stress development. However, in blood plasma, the level of active iron increases, leading to heightened oxidative stress and a decrease in antioxidant enzyme levels. Towards the conclusion of the experiment, the antioxidant blood system levels stabilize.

Key words: *adrenaline, hemoxygenase, non-heme iron, myocardium, oxidative stress.*

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ДИНАМІКА ВМІСТУ НЕГЕМОВОГО ЗАЛІЗА В МІОКАРДІ ТА АКТИВНІСТЬ ГЕМОКСИГЕНАЗИ В УМОВАХ ГІПЕРЕРГІЧНОГО ВПЛИВУ

С. П. Бесчасний, С. К. Семенюк

Ферум є головним елементом транспорту газів кров'ю, входить до складу антиоксидантних ферментів клітини. Вільне залізо, яке не є зв'язаним з білками або іншими молекулами, може бути хімічно активним і відігравати різні ролі у клітинах та тканинах. Однак, його висока хімічна активність може також призводити до утворення вільних радикалів, що може пошкодити клітини та спричинити окислювальний стрес.

В метаболізмі серця, де вільні радикали та окислювальний стрес можуть бути шкідливими, контроль за вільним залізом є дуже важливим. Організм використовує різні механізми для керування рівнем вільного заліза, такі як залізозв'язуючі білки та антиоксиданти, щоб забезпечити правильний баланс і запобігти можливим пошкодженням.

Досліджували динаміку вмісту негемового заліза в міокарді в умовах «адреналінового удару». На початку, в середині та наприкінці експерименту, визначали кількість негемового заліза у міокарді та плазмі крові. Одночасно з'ясовували вміст малонового діальдегіду, дієновихкон'югатів, супероксиддисмутази, каталази, відновленого глутатіону та активність гемоксигенази.

Вміст негемового заліза у міокарді до кінця експерименту знижувався на тлі посилення активності гемоксигенази-1. Разом з тим, рівень негемового заліза у плазмі крові збільшувався. Одночасно збільшилися показники маркерів оксидативногостресу у плазмі крові – малонового діальдегіду та дієнових кон'югатів. Рівень антиоксидантного ферменту супероксиддисмутази знижувався, під кінець експерименту спостерігалося відновлення його рівня. Подібна тенденція спостерігалася при визначення рівня відновленого глутатіону. Зазначені результати вказують на те, що в умовах метаболічного пошкодження міокарду відбувається активізація гемоксигеназного захисту від надмірного накопичення негемового заліза що захищає серце від розвитку оксидативного стресу. Проте, у плазмі крові збільшується рівень активного заліза, яке призводить до посилення оксидативного стресу в крові, відбувається підвищення вмісту маркерів оксидативного стресу на тлі зниження показників антиоксидантних ферментів. Під кінець експерименту відбувається стабілізація рівня антиоксидантної системи крові.

Ключові слова: *адреналін, гемоксигеназа, негемове залізо, міокард, оксидативний стрес.*

Introduction

Changes in iron metabolism in cardiomyocytes can have serious implications for heart function and overall cardiovascular health. Iron plays a dual role in oxidative stress: it can act as a source or as an object of oxidative damage (Sies, 2015; Bozzaet et al., 2020). Iron participates in reactions that lead to the formation of free radicals, in particular hydroxyl. These radicals can cause damage to cells by oxidation of lipids, proteins and nucleic acids (Aebi, 1984). Sources of iron for such reactions can be deposited free iron in cells or tissues, as well as its inadequate binding to proteins (such as transferin and ferritin). Also, the enzyme can catalyze the formation of free radicals by the Fenton reaction, which subsequently leads to increased oxidative stress. Iron acts as a co-factor in proteins involved in basic biological processes, in particular in oxidative metabolism, storage and transport of oxygen. Iron plays an important regulatory role in cell biology; however, excessive levels of intracellular iron are toxic (Khan et al., 2011).

It should be noted that oxidative stress in the body is usually regulated by antioxidant mechanisms that protect cells from free radical damage. When the balance between the production of free radicals and antioxidant mechanisms is disturbed, an imbalance may occur, which leads to oxidative stress and cell damage (Bozzaet et al., 2020; Sokolenko & Sokolenko, 2020).

Non-heme iron is a source of redox-active iron that can participate in the Fenton reaction to form toxic free hydroxyl radicals. The toxicity of lipophilic heme is enhanced by its ability to integrate into the hydrophobic phospholipid layer of cell membranes. As a result, oxidation of cell membrane components is enhanced, which promotes the formation of cytotoxic lipid peroxides. In the future, it increases the permeability of membranes and increases lysis and cell death (Ballaet et al., 2005; Rother et al., 2005; Kumar, 2005).

Non-heme iron also stimulates leukocyte activation and migration, expression of cell adhesion molecules, induction of pro-inflammatory cytokines and acute phase proteins (Chiabrando et al., 2014; Bozzaet et al., 2020). Especially these processes are dangerous to the cardiovascular system.

Deposition of iron in the myocardium occurs through L-type calcium channels and through endosomes (Stamenkovicet et al., 2019; Fuhrmann & Brüne, 2022). First, the iron accumulates in the myocardium of the ventricles and later in the atria. Deposition of iron in myocardial cells occurs in the form of ferritin and hemosiderin (Santambrogio et al., 2007). These are the two main forms in which iron is stored in the body. Both forms can cause tissue damage through the formation of free radicals, potentially leading to cardiomyopathy and other heart diseases (Theil, 2013). Ferritin is a protein complex that serves as the primary intracellular protein for iron storage and releases it in a controlled manner. Hemosiderin is a less soluble form of iron storage, which is produced as a result of ferritin breakdown. The accumulation of hemosiderin can occur with iron overload or hemochromatosis (Santambrogio et al., 2007).

Antioxidant reserves of cells are not unlimited, free radicals accumulate, lipid peroxidation, and cell membrane damage are triggered. Thus, myocardial overload by the enzyme causes myocardial dysfunction (Wagener et al., 2001; Beschasnyi, 2022).

The purpose of this study was to find out how the dynamics of non-heme iron change under conditions of hyperergic effects on the myocardium.

Material and methods

Outbred male laboratory mice at the age of 8 weeks were used in the experiments. At this age, the physiological parameters of mice are more stable, which is important for obtaining reliable and reproducible results. Additionally, 8-month-old mice are approximately equivalent to a human age of 30–40 years, making the results more relevant for application to humans (Paulter, 2004). The animals were kept in plastic cages with a 12-hour light regime, they had free access to food and water. Mice were randomly divided into two groups (n = 15 in each group). Metabolic myocardial infarction (MMI) was simulated in an experimental group of animals by intracranial epinephrine administration (1 mg/kg) for 4 hours (Todd et al., 1985). Animals in the control group were injected with an equivalent volume of saline.

In plasma and myocardium on the first, seventh and fourteenth days, the content of non-heme iron (NHI) was determined using a colorimetric analysis based on the reaction of non-heme iron with a batofenanthropin reagent (Duarte & Neves, 2022).

The determination of malondialdehyde (MDA) was carried out spectrophotometrically. To perform the analysis, blood plasma was diluted with 0.1-molar phosphate buffer (pH 7.4) and incubated for 10 minutes. Then, a 1-molar solution of potassium permanganate and a 10-molar solution of ferric oxide were added, followed by a reaction with 2-thiobarbituric acid (Draperet et al., 1993; Tsikas, 2017).

To determine the content of diene conjugates (DC), 4 ml of a heptane-isopropanol mixture (1:1) was added to 0.2 ml of blood plasma and mixed for 30 minutes in a laboratory mixer. The separation of the heptane and aqueous isopropanol phases was achieved by adding a hydrochloric acid solution. After settling and stratification of the mixture, a heptane layer was selected, in which acetyl hydroperoxides were measured by the degree of light absorption at a wavelength of 233 nm (Blair, 2006).

Determination of superoxide dismutase (SOD) activity in red blood cells was performed by spectrophotometric method (Babior & Kipnes, 1976; Bannister et al., 1987). Catalase activity was also determined spectrophotometrically, by the ability of ammonium molybdate to form a stable colored complex with hydrogen peroxide (Aebi, 1984). Reduced glutathione (GSH) was determined by glutathione peroxidase activity using hydrogen peroxide as a reducing substrate and Elman reagent (Blair, 2010). Hemoxygenase (NO-1) activity was determined using methemalbumin incubation medium, NADPH and phosphate buffer to which supernatant from homogenate was added (King et al., 1978).

The results of the study were processed using the statistical package of the license program “STATISTICA 6.0” (StatSoftInc., USA). The normality of the distribution of indicators was established according to the Shapiro-Wilk criterion. Descriptive statistics are presented as arithmetic mean and standard deviation – M ± SD. Comparison of indicators was carried out using the non-parametric Kraskell-Wallis method, followed by pairwise comparison of groups using the non-parametric Mann-Whitney test. Differences were considered significant at $p < 0.05$.

Results and discussion

Damage to an abnormally high number of red blood cells leads to the release of hemoglobin, which is a powerful prooxidant, a significant amount of iron accumulates in the blood (Bettiol et al., 2022). This is especially evident during ischemic damage

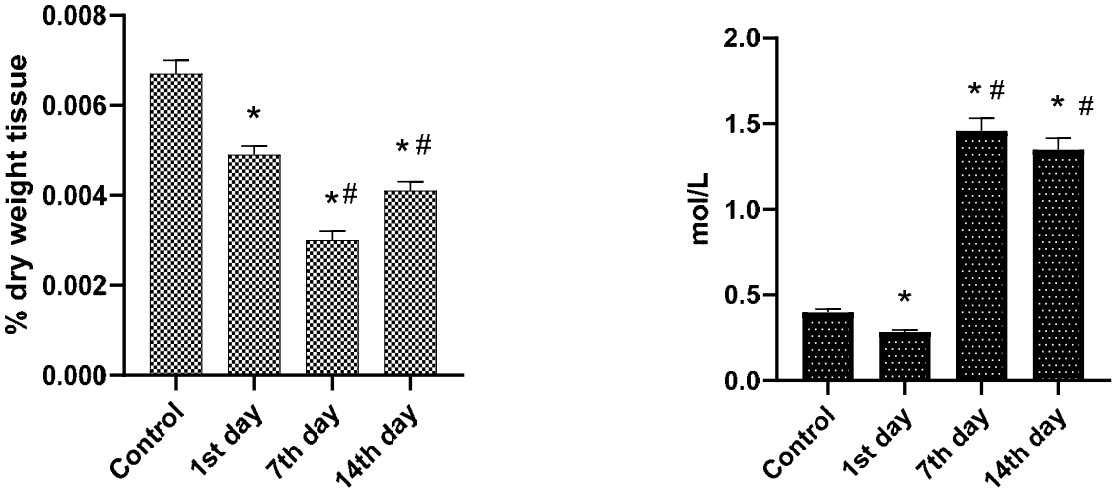
to the heart, intravascular hemolysis. The results of the study showed that the content of non-heme iron in the heart tissue decreased during the fourteen days of the experiment. The lowest level was set on the seventh day of the experiment (Fig. 1-A). Compared with the control (0,007 ± 0,003%) – the level of non-heme iron decreased more than twice (0,003 ± 0,0002% of dry weight tissue). At the end of the experiment, the NHI content began to increase slightly (0,004 ± 0,0002%), which is probably due to the activity of the hemoxygenase system.

The NHI content in plasma differed from the NHI content in the myocardium. On the seventh day, the level of NHI increased to a maximum level of 1.46 ± 0.073 mol/L (at the start of the experiment it was 0.399 ± 0.019 mol/L). At the end of the experiment, the indicator was also high – 1.349 ± 0.063 mol/L (Fig. 1-B).

MDA is known to be a reactive compound, which can damage cellular structures and functions. Such lesions can play a role in the development of various diseases, such as cardiovascular diseases, diabetes, chronic kidney diseases and others. An increase in the level of MDA in the body can serve as a sign of oxidative stress and may indicate the presence of pathological processes. MDA level measurement is used as a biomarker of oxidative damage in the body (Ayala et al., 2014). Interesting were the results of measuring the level of MDA and diene conjugates in animals with metabolic myocardial infarction (Fig. 2).

The level of MDA in the blood increased maximally on the seventh day (12,87 ± 0,64 mmol/l) after the metabolic myocardial infarction (Fig. 2.A). On the fourteenth day, the level of MDA did not differ from the previous indicator (13,41 ± 0,67 mmol/l). Regarding the level of diene conjugates – at the beginning of the experiment there was no significant increase in the level (1.17 ± 0.05 OD/ml) compared to control (1.08 ± 0.04 OD/ml) of these by-products of lipid peroxidation (Fig. 2.B). However, on the seventh (1.35 ± 0.06 OD/ml) and fourteenth days (1.53 ± 0.06 OD/ml), there was an increase in the level of diene conjugates, which is consistent with an increase in the level of MDA.

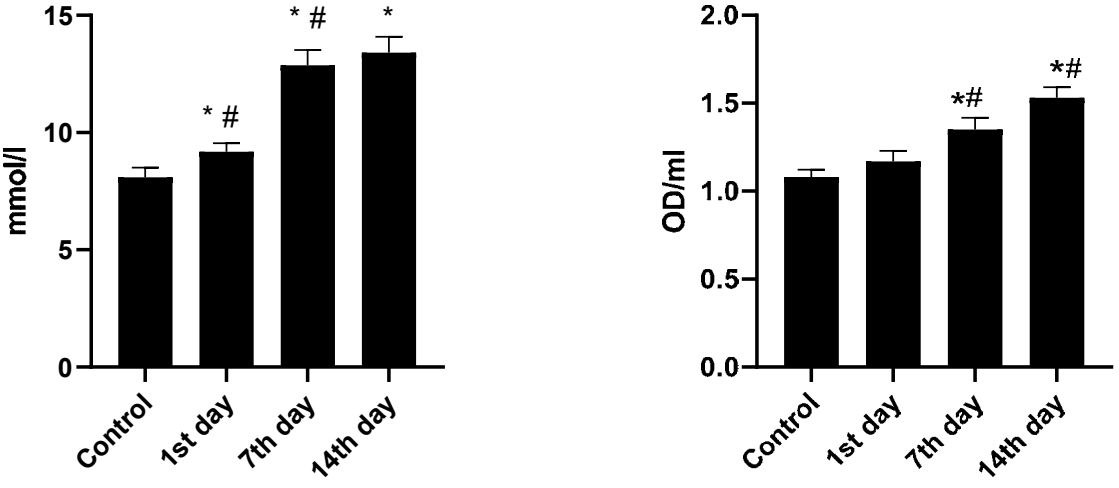
The activity of superoxide dismutase by the seventh day of the experiment was significantly ($p \leq 0,05$) reduced (2.61 ± 0.13 units/ml), but at the end of the experiment, it almost returned to the level of control (3.42 ± 0.17 units/ml) (Fig. 3-A). Catalase indicators were somewhat different. At the beginning of the experiment, there was a decrease in its activity (5.22 ± 0.26 mol/L/min). On the seventh day, the activity increased (9.18 ± 0.45 mol/L/min), but at the end of the experiment, it decreased again in comparison with the control (6.03 ± 0.3 mol/L/min) (Fig. 3-B).



A B

Fig. 1. Dynamics of non-heme iron content in heart tissue (A) and blood plasma (B) under conditions of metabolic myocardial infarction

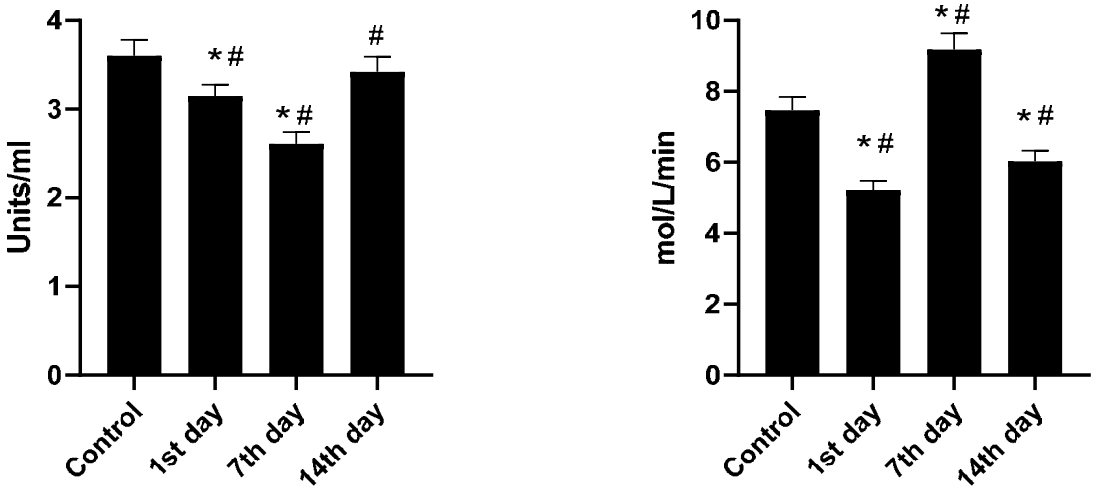
*Notes: * – significant differences from the corresponding indicators in the control group, $p \leq 0,05$; # – significant differences from the corresponding indicators from the previous period, $p \leq 0,05$*



A B

Fig. 2. Plasma levels of malonic dialdehyde (A) and diene conjugates (B) under hyperergic stimulation

*Notes: * – significant differences from the corresponding indicators in the control group, p≤0,05; # – significant differences from the corresponding indicators from the previous period, p≤0,05*



A B

Fig. 3. Activity of superoxide dismutase (A) and catalase (B) in blood plasma under the influence of hyperergic stimulation

*Notes: * – significant differences from the corresponding indicators in the control group, p≤0,05; # – significant differences from the corresponding indicators from the previous period, p≤0,05*

Interesting was the dynamics of reduced glutathione (Fig. 4). In the first days after MIM, there was a decrease in its level (3.87 ± 0.15 mg/ml) in red blood cell hemolysate (compared with the control). On the seventh and fourteenth days (6.39 ± 0.31 mg/ml and 5.85 ± 0.29 actually), its contents began to recover (compared to the first day of the experiment), but did not reach the level of control (9.54 ± 0.47 mg/ml).

Regarding the activity of hemoxygenase (NO-1) – in the first days after MIM, its level did not increase. On the seventh day, there was an increase in NO-1 activity

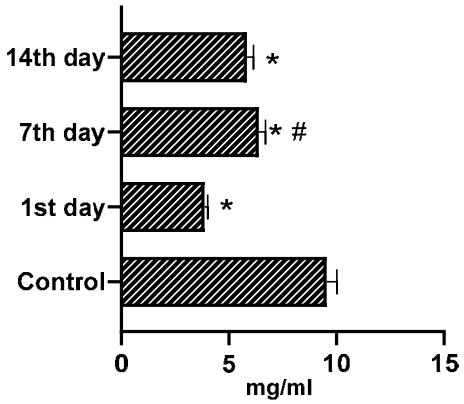


Fig. 4. Reduced glutathione content in red blood cell hemolysate of the study groups

*Notes: * – significant differences from the corresponding indicators in the control group, p≤0,05; # – significant differences from the corresponding indicators from the previous period, p≤0,05*

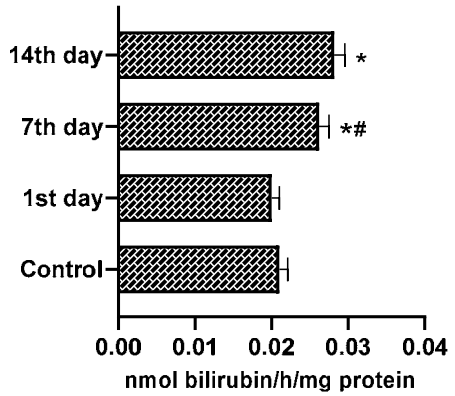


Fig. 5. Hemoxygenase activity in the myocardium of the study groups

*Notes: * – significant differences from the corresponding indicators in the control group, p≤0,05; # – significant differences from the corresponding indicators from the previous period, p≤0,05*

(0.026 ± 0.001 nmol bilirubin/h/mg protein), which lasted until the end of the experiment (0.028 ± 0.001 nmol bilirubin/h/mg protein) (Fig. 5). At the same time, a significant increase occurred on the 7th day of the experiment. The results obtained are consistent with a decrease in the level of non-heme iron in the myocardium and an increase in its level in the blood.

It is known that "free" iron has pro-inflammatory properties, in particular, it causes the activation and migration of leukocytes, the formation of reactive oxygen species, and increases vascular permeability (Wageneret et al., 2001; Graça-Souza et al., 2002). Heme also acts as a chemotactic molecule for neutrophils, inducing the formation of leukotriene B4 (Monteiro et al., 2011). Some studies indicate the participation of the non-heme enzyme in the development of heme-associated dysfunction of the endothelium and atherosclerosis (Müllebner et al., 2015; Miguel et al., 2021; Bettiolet et al., 2022).

Under the conditions of MIM, the content of non-heme iron in the heart tissue is reduced (up to the level of 0,003 ± 0,0002% of dry weight tissue) against the background of its increased level in blood plasma (1.46 ± 0.073 mol/L). At the same time, the level of malonic dialdehyde (12,87 ± 0,64 mmol/l) and diene conjugates (1.53 ± 0.06 OD/ml) in blood plasma increased, indicating the development of oxidative stress.

The development of oxidative stress is also evidenced by a decrease in the activity of antioxidant enzymes superoxide dismutase (2.61 ± 0.13 units/ml) and catalase (5.22 ± 0.26 mol/L/min). A decrease in the activity of reduced glutathione is also consistent with this (3.87 ± 0.15 mg/ml). However, at the end of the experiment, the adaptation of enzyme systems occurs – superoxide dismutase restores its activity up to the level of 3.42 ± 0.17 units/ml.

Hemohxygenase is known to play an important role in protecting cells from excessive amounts of non-heme iron. In particular, HO-1 protects mitochondria that are susceptible to hemin (Müllebneret et al., 2015). It is proved that HO-1 protects cells from ferroptosis. This can explain the increase in HO-1 activity in the myocardium, especially during the middle and end of the experiment. Surely the increase in HO-1 activity leads to an increase in the level of non-heme iron in the blood plasma, which is protective. Thereby, the protection of cardiomyocytes from ferroptosis is realized.

Prospects for further research is to study the participation of hemoxygenase in the development of the process of myocardial ferroptosis.

Conclusions

Under the conditions of metabolic myocardial infarction, there is an increase in the level of non-heme iron in blood plasma against the background of a decrease in its level in the myocardium.

The decrease in the level of non-heme fermium in the myocardium is the result of the activation of hemoxygenase, which performs a protective antioxidant function, protecting the myocardium from the development of ferroptosis, overload from non-heme iron.

"Adrenaline kick" at the beginning of the experiment causes a decrease in the activity of antioxidant enzymes superoxide dismutase and catalase, a decrease in the level of reduced glutathione. However, by the end of the experiment, there was a restoration of the activity of these enzymes and a partial increase in the level of reduced glutathione.

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