HORMETIC EFFECT ON THE TOXIC ACTION OF COPPER IONS DEPENDS ON THE TISSUE DISTRIBUTION OF COPPER IONS

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Summary. The role of inter-tissue localization of copper ions in the processes of hormesis to copper sulfate was investigated. The ability of the liver, spleen, heart, brain, and kidneys to bind copper ions was determined in cases of lethal outcomes after a single administration of copper sulfate to animals (Wistar rats) and in cases of hormesis to this compound. Copper ions in the tissues were determined by atomic absorption spectrometry. It was shown that the hormetic effect depends on the inter-tissue distribution of copper ions, and a relatively large amount of copper can be deposited by serum proteins.

Keywords: Copper, resistance, hormesis, Wistar rats, copper sulfate, fibrosis.

1. INTRODUCTION

Preliminary exposure of animals to relatively small (adaptive) doses of copper sulfate is accompanied by the development of resistance (resistance) of animals to subsequent exposure to large and even lethal doses of copper sulfate [1]. Although the phenomenon of hormesis has long been known, and its role in adaptation mechanisms, including the formation of resistance to drugs, can be attributed to one of the most important biomedical issues, with a significant amount of research dedicated to its resolution, it is far from being completely deciphered. The relevance of the mechanisms of hormesis is beyond doubt, as this knowledge has fundamental theoretical value in understanding the mechanisms of adaptation and no less practical value in medicine.

An important stage in the study of hormesis mechanisms is the selection or development of an adequate model of this phenomenon. We believe that such a model can serve as a model of hormesis to the toxic action of copper ions [1]. Firstly, copper ions are essential and exhibit toxicity at high doses, and the organism is capable of developing hormesis to them. Secondly, the mechanisms of copper ion action are relatively well-studied. Thirdly, the concentrations and localization of copper ions in the body and cells can be easily determined. Based on the relevance,
practical significance, and justification of the experimental approach, we determined the intertissue and intracellular localization of copper ions in animals in the case of hormesis formation.

2. METHODS

The experiments were conducted on adult male rats weighing 150-200 grams of the Wistar strain. To induce hormesis, experimental animals were administered copper sulfate three times at a dose of 1 mg/100g of body weight with a 48-hour interval between administrations, as described in our previous work [1]. The animals were divided into three groups, to which lethal doses of copper sulfate were administered in different schemes:

1. Administration of only a large dose of copper sulfate, 2.5 mg/100 g of body weight.
2. Three successive administrations of small doses of copper sulfate (1 mg/100 g of body weight) with a 48-hour interval between administrations, followed by the administration of a large dose of 2.5 mg/100 g of body weight.
3. Three successive administrations of small doses of copper sulfate (1 mg/100 g of body weight) every 48 hours, followed by a 30-day break, followed by three more successive administrations of adaptive doses, and then the administration of a large dose of 2.5 mg/100 g of body weight after 24 hours.

Copper ions were determined in the serum, liver, spleen, heart, brain, and kidneys using atomic absorption spectrophotometry (SELMI C-600), as described in the work [2,3]. The results were expressed in micrograms of copper ions per gram of dry tissue.

3. RESULTS

It was found that 45 minutes after a single intraperitoneal injection of a large dose of copper sulfate (2.5 mg/100 g), the liver contained 9.5 micrograms of copper ions per gram of tissue (Figure 1).

![Graph showing copper ion content in various tissues](image-url)

*Fig. 1. The total content of copper ions in all examined tissues (1), in the liver (2), spleen (3), heart (4), brain (5), and kidneys (6) 45 minutes after the administration of a large dose of CuSO$_4$·5H$_2$O (2.5 mg per 100 g of body weight). Presented are the mean values from 3 independent experiments and standard errors.*
Under these conditions, the spleen had a copper ion content of 10.3 micrograms per gram, which was the same as in the liver. At the same time, the content of copper ions in the heart and brain was 3.5 to 3.7 times lower than in the liver and spleen (Figure 1). The highest copper content was detected in the kidneys, which amounted to 28.5 micrograms per gram of tissue (Figure 1).

Such a high content of copper ions in the kidneys (3 times higher than in the liver) may indicate a rapid excretion of copper ions from the body after a single intraperitoneal injection of a lethal dose, even 45 minutes after its administration. It cannot be ruled out that the death of animals at such doses may occur as a result of kidney function suppression (overload) and, consequently, intoxication of the body [2]. It is worth noting that 45 minutes after a single administration of a dose of 2.5 mg/100g to the experimental animals, these 5 examined tissues (liver, spleen, heart, brain, and kidneys) collectively contained 53.89 micrograms per gram of dry tissue (Figure 1), and in terms of quantitative indicators, copper ions were distributed in the following sequence: kidneys > spleen = liver > heart = brain.

In order to determine the interconnection between the nature of intertissue distribution of copper ions and the formation of resistance to a lethal dose, a series of experiments on the copper ion content in the examined tissues was conducted after three sequential administrations of copper sulfate followed by the administration of a lethal dose to the animals. In other words, the administration scheme that ensured hormesis formation was used.

It was found that the concentrations of copper ions in the examined organs varied differently compared to a single administration of a large dose of copper sulfate. Specifically, the total copper content in all examined organs increased by 2.6 times compared to a single administration of the large dose. However, the copper content in the liver decreased by 16%, in the spleen decreased by 21%, in the heart, on the other hand, increased by 2.8 times, and in the kidneys, it was increased by almost 4 times (3.97) (Figure 2).

The obtained data demonstrate that under this administration regimen, the highest copper content is localized in the kidneys, similar to the animals that were administered only a large dose. However, in this case, more than 80% of the total copper content in all examined tissues was accounted for by the kidneys (Figure 2). Based on this, it can be concluded that three sequential administrations of copper sulfate at relatively small doses over a period of 5 days may have resulted in adaptive changes not only at the level of liver functional adaptations but also in the kidneys. These changes could potentially lead to a significant increase in their ability to accumulate and excrete copper ions, preventing the organism from experiencing toxicity compared to a single administration of copper sulfate, even at a lower cumulative dose (2.5 mg instead of 3 mg/100 g in the case of fractionated administration). However, this assumption would require further experimental verification.

One of the important and unresolved issues in the problem of hormesis is the question of the duration of its persistence after the removal of active inducers or the possibility of forming metabolic memory.

Previously, it was shown that 30 days after three consecutive administrations of copper sulfate to experimental animals, the copper content in their livers did not
Fig. 2. Copper ion content in the examined tissues (1), liver (2), spleen (3), heart (4), brain (5), and kidneys (6) after three sequential administrations of adaptive doses (1 mg per 100 g of body weight) of CuSO4*5H2O with a 48-hour interval between administrations, followed by the administration of a large dose (2.5 mg per 100 g of body weight) 45 minutes before measurement. Presented are the mean values from three independent experiments and their standard errors.

...differ from that in intact control animals [2]. Additionally, it was demonstrated that if these animals were administered lethal doses of copper sulfate one month later, they also exhibited hormesis, albeit to a lesser extent compared to animals that received the lethal dose 24 hours after the administration of small doses of this toxin [1,2]. However, it remained unclear what the nature of the interorgan distribution of copper ions was in animals that retained resistance to the lethal dose one month after induction. Knowledge of the answer to this question is pivotal in understanding the mechanisms of potential metabolic memory. Therefore, in the next series of experiments, the nature of interorgan localization of copper ions was determined after the administration of a large dose of copper sulfate (2.5 mg/100 g of body weight) 30 days after these animals received adaptive doses of copper sulfate.

It was found that if animals were administered a large dose of copper sulfate 30 days after they had received small doses three times, the amount of copper ions in the liver, spleen, and brain remained the same as after a single administration of only the large dose (Figure 1, 3). Additionally, the copper ion content increased by 67% in the heart, by 3 times in the kidneys, and the total content across all examined organs was 2 times higher compared to a single administration of the large dose of copper sulfate (Figure 3).

From these data, two conclusions can be drawn. Firstly, after a month, copper ions were not excreted from all organs, and secondly, the prolonged retention of copper ions in the examined organs did not correlate with lethality. It can be hypothesized that the key factor in the formation of resistance in the organism to copper ions depends not so much on the quantity of copper in the body but on the...
Fig. 3. The copper ion content in the examined tissues (1), liver (2), spleen (3), heart (4), brain (5), and kidneys (6) after three successive administrations of adaptive doses (1 mg per 100 g of body weight) of CuSO4*5H2O with a 48-hour interval, followed by a repeat administration of adaptive doses 30 days later, and subsequent administration of a large dose after 24 hours. Presented are the mean values from three independent experiments and their standard errors.

rate of their accumulation in the body. When toxic copper ions accumulate relatively slowly in the body, the formation of a "neutral" pool of these ions may occur, and such a metabolic system restructuring may function even at high concentrations of copper ions, thereby providing resistance.

In connection with this, it was of interest to determine the copper ion content in the blood serum of experimental animals after different schemes of copper sulfate administration. It was found that after a single administration of copper sulfate in a large dose, 45 minutes after its administration, 4.9 micrograms of copper ions per milligram of protein were detected, which was several times higher than the reference values (Figure 4). In the case where animals were previously administered small adaptive doses of copper sulfate and then received a large dose 24 hours later, copper levels in the blood serum were measured at up to 11 micrograms per gram of protein after 45 minutes (Figure 4), which corresponded to the total administered dose of copper sulfate.

The results from the following series of experiments yielded some unexpected findings. Specifically, the copper ion content in the blood serum after a one-month interval between the administration of small and large doses was 28 micrograms per milligram of protein, which was 5.5 times higher than after a single administration of only the large dose and even 2.5 times higher than in the case of sequential administrations of small and large doses with a 24-hour interval (Figure 4).

These results suggest that the copper ion content in the serum depends on the administration scheme into the organism and it can be retained by serum components for a considerable duration.
Fig. 4. The copper ion content in the blood serum of animals in the case of a single administration of copper sulfate in a large dose - 2.5 mg/100 g of body weight (1), in the case of three successive administrations of small doses (1 mg/100 g of body weight) followed by the administration of a large dose - 2.5 mg/100 g after 24 hours of the small dose (2), and in the case of three successive administrations of small doses (1 mg/100 g of body weight) followed by a repeat administration of small doses after 30 days and subsequent administration of a large dose after 24 hours (3). Presented are the mean values from three independent experiments and their standard errors.

4. CONCLUSION
There is no direct correlation between the copper ion content in vital organs and lethality. The occurrence of death (poisoning) after the administration of copper sulfate to animals depends not so much on the dose as on the administration scheme, specifically on the time intervals between administrations. It can be assumed that the discrete introduction of copper ions into the organism, i.e., the lethal dose (2.5-3.0 mg/100 g) administered three times at one mg per 100 g with a 48-hour interval between administrations, triggers metabolic changes that provide resistance to the subsequent action of lethal doses of this toxin.

References: