ORIGINAL PAPER
ORYGINALNY ARTYKUŁ NAUKOWY

Health Prob Civil. 2022, Volume 16, Issue 4
ISSN 2353-6942

INFLUENCE OF UNITHIOL ON THE LIVER IN THE CONDITIONS OF A MECHANICAL INJURY AGAINST THE BACKGROUND OF POISONING BY ZINC AND COPPER SALTS

WPŁYW UNITHIOLU NA WĄTROBĘ W WARUNKACH URAZU MECHANICZNEGO NA TLE ZATRUCIA SOLAMI CYNKU I MIEDZI

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Authors' contribution
Wkład autorów:
A. Study design/planning
zaplanowanie badań
B. Data collection/entry
zebranie danych
C. Data analysis/statistics
dane – analiza i statystyki
D. Data interpretation
interpretacja danych
E. Preparation of manuscript
przygotowanie artykułu
F. Literature analysis/search
wyszukiwanie i analiza literatury
G. Funds collection
zebranie funduszy

Summary

Background. The aim of the study was to find out the influence of excess copper and zinc ions in drinking water on the bile-forming and bile-secreting functions of the liver in the conditions of mechanical trauma and the effectiveness of the antidote therapy.
Material and methods. The experiments were performed on 60 Wistar strain male rats weighing 180-200 g. There were 3 groups of experimental animals in the experiment: 1 group – only skeletal trauma was simulated; 2 group – a skeletal injury was simulated and zinc and copper salts were administered; 3 group – skeletal trauma + zinc and copper salts followed by Unithiol treatment.
Results. In the late period of traumatic illness (14-28 days), after a severe skeletal injury, the intensity of bromsulfalein increased in the liver tissue, and there were signs of increased formation of direct bilirubin in terms of additional intake of copper and zinc ions. The animals with severe skeletal trauma were accompanied by a significant deterioration in the functional state of the liver in comparison to the animals with similar skeletal trauma without additional intoxication.
Conclusions. Under conditions of additional intake of copper and zinc ions (in the 2nd group), after 14 days and 28 days of observation, all the studied indicators are much higher than in the group of the animals without additional intoxication with these ions. The use of Unithiol reduced the toxic effect of copper and zinc ions on the studied indicators of the functional state of the liver both in the early and late period of the traumatic disease.
Keywords: Unithiol, zinc sulfate, copper sulfate, bile acids, injury, wounds

Streszczenie

Wprowadzenie. Celem pracy było poznanie wpływu nadmiaru jonów miedzi i cynku w wodzie pitnej na funkcje wytwarzania i wydzielania żółci w wątrobie w warunkach urazu mechanicznego oraz skuteczności terapii antidotum.
Materiał i metody. Doświadczenia przeprowadzono na 60 samcach szczurów szczepu Wistar o masie 180-200 g. W badaniu uczestniczyły 3 grupy zwierząt doświadczalnych: grupa 1 – symulowano tylko uraz szkieletowy; grupa 2 – symulowano uraz szkieletowy i podawano sole cynku i miedzi; grupa 3 – uraz szkieletowy + sole cynku i miedzi, a następnie leczenie Unithiolem.
Wyniki. W późnym okresie choroby urazowej (14-28 dni), po ciężkim urazie szkieletowym, wzrosło nasilenie bromosulfaleiny w tkance wątrobowej i wystąpiły objawy zwiększonego wytwarzania bilirubiny bezpośredniej na skutek dodatkowego przyjmowania jonów miedzi i cynku. Zwierzętom z ciężkim urazem szkieletowym towarzyszyło znaczne pogorszenie stanu czynnościowego wątroby w przeciwieństwie do zwierząt z podobnym urazem szkieletowym bez dodatkowego zatrucia.
Wnioski. W warunkach dodatkowego przyjmowania jonów miedzi i cynku (w drugiej grupie), po 14 i 28 dniach obserwacji, wszystkie badane wskaźniki były znacznie wyższe niż w grupie zwierząt bez dodatkowego zatrucia jonami. Stosowanie Unithiolu zmniejszyło toksyczny wpływ jonów miedzi i cynku na badane wskaźniki stanu czynnościowego wątroby zarówno we wczesnym, jak i późnym okresie choroby urazowej.
Słowa kluczowe: Unithiol, siarczan cynku, siarczan miedzi, kwasy żółciowe, uraz, rany

Tables: 3
Figures: 0
References: 20
Submitted: 2022 Jul 18
Accepted: 2022 Nov 2

Melnyk N, Fedoriv O, Kopach O. Influence of Unithiol on the liver in the conditions of a mechanical injury against the background of poisoning by zinc and copper salts. Health Prob Civil. 2022; 16(4): 323-329. https://doi.org/10.5114/hpc.2022.120823

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Introduction

In modern conditions, heavy metal salts are among the main environmental pollutants in Ukraine. Whereas in the industrial regions of Ukraine (central, southern and eastern) the soil and water are contaminated predominately by cadmium (Cd), lead (Pb) and ^{88}Strontium (Sr) [1-2], then zinc (Zn) and copper (Cu) predominate almost throughout the whole of Ukraine [3-4]. Even in such an ecologically safe region as Ternopil, there is an excessive accumulation of copper and zinc ions in living organisms inhabiting reservoirs [5]. In the drinking water in Ternopil it exceeds by a factor of 20 the maximum allowable concentration of copper ions and by a factor of 3 the allowable maximum of zinc ions, as evidenced by the Environmental Monitoring Program of Ternopil region for 2006-2010 [6-7].

A number of publications present the mechanisms of adverse effects of the accumulation of copper and zinc ions on the body. There is a pronounced interference of these ions in the biliary and biliary systems. There are significant changes in the functional state of the liver, pancreas, muscles, and transmembrane processes [8-9]. Therefore, a hypothesis arose about the possibility of modifying the course of various pathological processes in the conditions of copper and zinc ions accumulation in the body, which required a special study.

As a model of pathology, we used severe skeletal trauma, the frequency of which is steadily increasing every year, and the effectiveness of treatment continues to be unsatisfactory [10-11]. In these conditions, there is a traumatic disease, which involves the pathological process of tissues and organs that are far from the area of direct damage, and is accompanied by the development of multi-organ dysfunction and insufficiency.

The aim of the study was to find out the influence of excess copper and zinc ions in drinking water on the bile-forming and bile-secreting functions of the liver in the conditions of mechanical trauma and the effectiveness of the antidote therapy.

Material and methods

The experiments were performed on 60 male Wistar rats weighing 180-200 g. Chronic intoxication with copper and zinc sulfates was performed by daily injection of the solutions with use of a probe into the stomach at a dose of 5 mg×kg^{-1} in terms of metal.

Skeletal trauma was modeled under thiopental-sodium anesthesia (40 mg×kg-1 body weight) by means of a dosed shock, which caused a closed fracture of both hips, and additionally modeled blood loss (20-22% of the volume of circulating blood) by crossing the femoral vein, which flowed into the paranephric tissue to form a hematoma [5,12].

All the experimental animals were divided into three groups. The control group included 10 intact animals.

The 1st experimental group consisted of 20 animals that simulated the skeletal injury itself.

In the 2nd group (20 individuals), skeletal trauma was simulated in conditions of chronic intoxication with copper and zinc sulfates after 14 days (n=10) and 28 days (n=10), respectively.

In the 3rd group (20 individuals), skeletal trauma was simulated in conditions of chronic intoxication with copper and zinc sulfates after 14 days (n=10) and 28 days (n=10), respectively. Also, Unithiol – a universal antidote to heavy metal salts in a dose of 0.01 g per 100 g of the animal's weight – was administered intraperitoneally to these animals for 2 weeks starting 1 day after the injury [8,13]. Unithiol forms mercaptans. These are complexes with heavy metals, combined with thiol enzymes (which helps to remove heavy metals from tissues).

After the end of the experiment, the animals were given thiopental-sodium anesthesia (60 mg·kg-1), bile was collected for 30 minutes, then a bromsulfalein test was performed, after which the animals were euthanized by the method of total bleeding from the heart. Bile, serum and liver tissue were taken for the study. Bile-forming and biliary function of the liver was assessed on the basis of guidelines for preclinical studies of drugs. After

anaesthetizing (sodium thiopental 60 mg×kg^{-1}) the animals, the common bile duct was catheterized and bile was collected for 30 min. In all the experiments, the catheter in the common bile duct was placed as standard in the same place, because the irritation of its proximal or distal part differently affects the intensity of bile secretion [14]. All the indicators were determined twice: in the early (after 14 days of skeletal trauma simulation) and the late period (after 28 days of skeletal trauma simulation) of the traumatic disease.

The experiment complied with the requirements of the European Convention for the Protection of Vertebrate Animals Used for Research and Other Scientific Purposes (Strasbourg, 1986) and the European Union Directive 2010/10/63 EU on animal experiments. The Commission on Bioethics of Ivan Horbachevsky Ternopil National Medical University, Ukraine, did not find any violations of moral and ethical norms during this study (Protocol No. 4 of October 2, 2019).

The obtained digital material was processed in the Department of System Statistical Surveys of Ivan Horbachevsky Ternopil State Medical University with the use of the software package STATISTICA (StatSoft Inc., USA) applying the parametric Student's test.

Results

As can be seen in Table 1, after 14 days, the content of total bile acids in the bile after the severe skeletal injury continued to be reduced compared with the control group (by 13.8%, $p<0.05$). After 28 days, the rate increased and reached the level of the control ($p>0.05$). Against the background of intoxication with copper and zinc salts, severe skeletal trauma led to an even greater decrease in the studied indicator in both observation periods. After 14 days it was 44.9%, and after 28 days – 35.6% less than in the control ($p<0.05$). During these observation periods, the content of total bile acids in the bile was also statistically significantly lower than in the group of the injured animals without additional intoxication with copper and zinc ions ($p<0.05$).

Table 1. The content of total bile acids in the bile in the late period of traumatic disease in the rats with excessive intake of copper and zinc ions and its correction by Unithiol (M±m)

Experimental conditions	14 days	28 days	*p*
Control = (2.25±0.10) g×L^{-1}			
1st group – skeletal trauma	1.94±0.08*	2.08±0.10	>0.05
2nd group – copper and zinc salts + skeletal trauma	1.24±0.07$^{*\#}$	1.45±0.07$^{*\#}$	>0.05
3rd group – copper and zinc salts + skeletal trauma + Unithiol	1.44±0.07$^{*\#}$	1.76±0.09$^{*\#o}$	<0.05

Notes: * – the differences in the control that are statistically significant ($p<0.05$); $^{\#}$ – the difference compared to the 1st group that is statistically significant ($p<0.05$); ^{o} – the difference compared to the 2nd group that is statistically significant ($p<0.05$).

The use of Unithiol contributed to the increase of the studied indicator in comparison with the group of the animals without correction: after 14 days – by 16.1% ($p<0.05$), after 28 days – by 21.4% ($p<0.05$). It should be noted that in this group, after 28 days, the indicator increased significantly compared to the previous observation period ($p<0.05$).

In turn, the content of direct bilirubin in the bile (Table 2) in the conditions of the skeletal injury after 14-28 days was normalized and did not differ significantly from the control group ($p>0.05$). The severe skeletal injury in conditions of additional intake of copper and zinc ions was accompanied by significantly greater violations: after 14 days the figure was 41.6%, and after 28 days – 37.7% less than in the control ($p<0.05$) and significantly lower than in the group of the animals with skeletal trauma without additional intoxication ($p<0.05$). The use of Unithiol in these conditions increased the formation of direct bilirubin. After 14 days, the rate was 25.0% higher than in the group of the animals without correction ($p<0.05$). After 28 days, this increase was 32.0% ($p<0.05$),

but the rate did not reach the level of the animals with the injury itself and was statistically significantly lower (p<0.05).

Table 2. The content of direct bilirubin in the bile in the late period of traumatic disease in the rats with excessive intake of copper and zinc ions and its correction by Unithiol (M±m)

Experimental conditions	14 days	28 days	p
Control = (67.58±3.68) μmol·L^{-1}			
1st group – skeletal trauma	62.58±3.36	64.03±2.61	>0.05
2nd group – copper and zinc salts + skeletal trauma	36.57±2.23$^{*\#}$	42.07±3.37$^{*\#}$	>0.05
3rd group – copper and zinc salts + skeletal trauma + Unithiol	45.70±2.50$^{*\#o}$	55.55±1.90$^{*\#o}$	<0.05

Notes: * – the differences in the control that are statistically significant (p<0.05); $^{\#}$ – the difference compared to the 1st group that is statistically significant (p<0.05); ^{o} – the difference compared to the 2nd group that is statistically significant (p<0.05).

One of the most sensitive and informative indicators of the functional state of the liver is the duration of bromosulfalein excretion in the bile. Our experiments showed that in the late period of traumatic illness (Table 3), after 14 days, the duration of bromsulfalein in conditions of the skeletal trauma was significantly longer than in the control (13.4%, p<0.05). After 28 days, the rate decreased and reached the level of the control (p>0.05). In the conditions of additional intake of copper and zinc ions, severe skeletal trauma caused a significantly longer duration of bromosulfalein release than in the control: after 14 days – by 76.3%, after 28 days – by 47.9% (p<0.05). In these conditions, the introduction of Unithiol led to an improvement in the studied indicator. Compared with the group of the animals without the correction, after 14 days, it was 10.6%, and after 28 days – 11.8% smaller, which was statistically significant. On the 28th day of the experiment, the duration of bromsulfalein release in the 2nd and 3rd studied groups significantly decreased compared to this indicator on the 14th day of the experiment (p<0.05).

Table 3. The duration of the bromosulfalein release in the late period of traumatic disease in the rats with excessive intake of copper and zinc ions and its correction by Unithiol (M±m)

Experimental conditions	14 days	28 days	p
Control = (37.33±1.43) min			
1st group – skeletal trauma	42.33±1.20*	39.83±1.61	>0.05
2nd group – copper and zinc salts + skeletal trauma	65.83±2.01$^{*\#}$	55.17±2.23$^{*\#}$	<0.05
3rd group – copper and zinc salts + skeletal trauma + Unithiol	58.83±1.83$^{*\#o}$	48.67±1.76$^{*\#o}$	<0.05

Notes: * – the differences in the control that are statistically significant (p<0.05); $^{\#}$ – the difference compared to the 1st group that is statistically significant (p<0.05); ^{o} – the difference compared to the 2nd group that is statistically significant (p<0.05).

Thus, in conditions of a severe skeletal injury, the normalization of the bromsulfalein release duration occurs after 28 days, and after 14 is much higher than in the control. Under the conditions of additional intake of copper and zinc ions (in the 2nd and the 3rd groups) after 14 days and 28 days of observation, all the studied indicators are much higher than in the group of the animals without additional intoxication with these ions.

The corrective use of Unithiol accelerates the elimination time of bromosulfalein in the bile, which is statistically significant compared to the animals without correction, but does not reach the level of the injured animals without additional intoxication with copper and zinc ions.

Discussion

Nowadays, in Ukraine under the conditions of war, there is a high mortality from injuries, accidents and poisoning, which mainly affects people of working age. It is known that traffic injuries are also significant in the

developed countries of Europe. Despite the tendency to decrease the indicators of general injuries, the incidence of victims with polytrauma is increasing and causes high mortality. On the other hand, the territory of Ukraine is significantly affected by adverse environmental factors, among which the leading place belongs to salts of heavy metals [15]. A clear confirmation of this is the excessive accumulation of copper and zinc ions in living organisms inhabiting water bodies of the Ternopil region, and exceeding the maximum permissible concentration of copper ions in the drinking water of the city of Ternopil by 20 times and zinc ions by 3 times.

To date, the key links in the pathogenesis of severe trauma have been established. Its course occurs in phases. At the first stage (early period of the traumatic disease), after the action of the traumatic factor, there is a significant activation of catabolic reactions with the development of energy deficit and processes of cellular intoxication. At the second stage (the late period of the traumatic disease), as a result of the exhaustion of the hypothalamic-pituitary-adrenal system, the accumulation of endotoxins and inflammatory mediators, the violation of immunological resistance and the addition of infection, secondary changes occur in the internal organs, which can lead to the development of multiple organ failure, which is considered the main cause of death [16].

At the same time, a number of publications indicate the mechanisms of adverse effects of the copper and zinc ions accumulation on the body, which describe significant changes in the functional state of the liver, pancreas, muscles, and transmembrane processes [17].

However, in the available literature, there are not enough reports on the pathogenetic features of disorders of the functional and morphological state of the liver against the background of a traumatic disease in the conditions of the accumulation of copper and zinc ions in the body [18]. A working hypothesis emerged that damage by ions of these heavy metals is an unfavorable background for the course of a severe injury. Their accumulation in the blood and other organs can affect the manifestations of multiple organ failure, change the duration of traumatic illness periods and require specific antidote treatment in complex intensive therapy, which required a special study.

So, during modeling of skeletal trauma, we established that in the late period of post-traumatic disease, severe liver dysfunction occurs. This is manifested by a significantly lower content of total bile acids and direct bilirubin in the bile after 14 days of the post-traumatic period. Also at this time, we recorded a slowdown in the release of bromsulfalein [19].

As for the second group of the experimental animals, which, in addition to skeletal trauma, were additionally injected with copper and zinc salts, we established a more significant impairment of liver function compared to the group of the animals with isolated skeletal trauma. A more significant impairment of liver function (as indicated by a decrease in the content of total bile acids and direct bilirubin, as well as a longer duration of the bromsulfalein action) in the animals with complex trauma is associated with the additional toxic effect of copper and zinc salts. This leads to more significant destruction of hepatocytes [20].

We also established that the use of an antidote (Unithiol) of heavy metal salts in the animals with combined trauma significantly reduces the toxic effect of copper and zinc ions. This is indicated by the improvement of liver function in the post-traumatic period.

Conclusions

1. Severe skeletal trauma causes dysfunction of the liver in the late period of traumatic disease, which is manifested by a significantly lower content of total bile acids and direct bilirubin in the bile, slowing the release of bromosulfalein after 14 days of the post-traumatic period. After 28 days, the indicators reach the level of control.

2. Under conditions of additional intake of copper and zinc ions, severe skeletal trauma is accompanied by a significant deterioration of the functional state of the liver: after 14 and 28 days the bile content of total bile acids and direct bilirubin, as well as the duration of bromosulfalein, is much longer than in the animals with similar skeletal trauma intoxication.
3. The use of Unithiol reduces the toxic effects of copper and zinc ion; the studied indicators of liver function after 14 and 28 days of the post-traumatic period are improved compared to the group of the animals without correction, but do not reach the level of the animals with skeletal trauma.

Disclosures and acknowledgments

The authors wish to acknowledge the contributions of Ivan Horbachevsky Ternopil National Medical University for assisting with data collection and to all the participants for providing information used in this study. The authors declare no conflicts of interest with respect to the research, authorship, and/or publication of this article. The research was funded by the authors.

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