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THE EFFECT OF COMBINED ACTION OF TYPES OF LEAD NANOPARTICLES AND STEARATES ON INDICATORS OF THE OXIDATIVE STRESS IN THE BODY OF EXPERIMENTAL ANIMALS

WPŁYW SKOJARZONEGO DZIAŁANIA RODZAJÓW NANOCZĄSTEK OŁOWIU I STEARYNIANÓW NA WSKAŹNIKI STRESU OKSYDACYJNEGO W ORGANIZMACH ZWIERZĄT DOŚWIADCZALNYCH

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

Summary

Background. The aim of this work was to determine the effect of the combined action of nanoparticles of stearates and lead on the state of lipid peroxidation, antioxidant defense, and the level of circulating immune complexes in experimental rats.
Material and methods. Experiments were conducted on four groups of white rats. The first (control) and second groups of animals consumed dechlorinated water from the city water supply. The third and fourth groups of animals consumed water, respectively, with the content of sodium stearate and potassium stearate in a dose of 1/250 LD_{50}. After 40 days of drinking the indicated waters, the animals of the second, third, and fourth groups were orally injected with lead nanoparticles at a dose of 1/250 of the LD_{50}.
Results. The combined effect of sodium stearate and lead nanoparticles, potassium stearate, and lead nanoparticles had the character of additive action and potentiation according to most indicators of lipid peroxidation and antioxidant protection. According to the change of the circulating immune complexes indicator in the blood, it had the character of antagonistic action.
Conclusions. The studies have shown that, in most indicators of lipid peroxidation and antioxidant system, the combined action of lead nanoparticles and stearates had the character of potentiation.

Keywords: lead nanoparticles, potassium stearate, sodium stearate, oxidative stress, water pollution

Streszczenie

Wprowadzenie. Celem pracy było określenie wpływu skojarzonego działania nanocząstek stearynianów i ołowiu na stan peroksydacji lipidów, obronę antyoksydacyjną oraz poziom krążących kompleksów immunologicznych u doświadczalnych szczurów.
Materiał i metody. Badania przeprowadzono na czterech grupach białych szczurów. Pierwsza (kontrolna) i druga grupa zwierząt spożywały odchlorowaną wodę z wodociągu miejskiego. Trzecia i czwarta grupa zwierząt spożywały wodę, odpowiednio z zawartością stearynianu sodu i stearynianu potasu w dawce wynoszącej 1/250 LD_{50}. Po 40 dniach picia wskazanych wód, zwierzętom z grupy drugiej, trzeciej i czwartej podano doustnie przez wstrzyknięcie nanocząstki ołowiu w dawce odpowiadającej 1/250 LD_{50}.
Wyniki. Zgodnie z większością wskaźników peroksydacji lipidów i ochrony antyoksydacyjnej, skojarzone działanie stearynianu sodu i nanocząstek ołowiu, a także stearynianu potasu i nanocząstek ołowiu, miało charakter efektu addytywnego i potęgowania. Zgodnie ze zmianą wskaźnika krążących kompleksów immunologicznych we krwi, miało to charakter działania antagonistycznego.
Wnioski. Badania wykazały, że w większości wskaźników peroksydacji lipidów i układu antyoksydacyjnego skojarzone działanie nanocząstek ołowiu i stearynianów miało charakter potencjalizacji.

Słowa kluczowe: nanocząstki ołowiu, stearynian potasu, stearynian sodu, stres oksydacyjny, zanieczyszczenie wody

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Introduction

Many modern consumer goods and products, such as some foods (chocolate, ice cream, and creams), detergents, perfumes and personal care products (toothpastes, sunscreens and foundations, lipsticks), varnishes, paints, clothing, footwear, household items contain micro- or nanostructures [1-4]. The main and very serious problem is that many substances that are completely safe in the usual bulk form, become extremely toxic and dangerous after grinding them to nanoscale [5-7]. In the form of nanoparticles, such materials acquire new, uncharacteristic properties. They enter the body by inhalation, through the mouth with water and food, especially through damaged skin [8], openings in cell membranes or by cellular transport mechanisms, as well as by medical and cosmetic means and can be distributed throughout the body. When inhaled, nanoparticles can penetrate the bronchi; from there, by synchronous movements of the cilia of the cells, they are sent up into the larynx and swallowed with saliva, water and food into the gastrointestinal tract [9-11]. Therefore, nanoparticles are the subject of research by many scientists around the world due to their unique chemical, physical, biological and pharmacological properties.

Another modern factor in environmental pollution is surfactants, including sodium and potassium stearates. The research literature data indicate a possible oral intake of surfactants and their penetration into the human body through intact skin [12]. Sodium stearate is used in cosmetics and pharmaceuticals as a detergent base for detergents, components of some types of soap, in shampoos, bath and shaving foams, hair dyes; as an additive in dry perfumes and deodorants and to toothpastes and cosmetic creams. Potassium stearate is used as an antiseptic (potassium soap), components of hand creams, shaving foam, cosmetic fragrances, as a basis for textile aids and other uses [13].

In real life, the biosystem is never affected by such factors alone. If certain characteristics of the biosystem change, it is always under the influence of a whole set of factors. Because stearates and lead in nanoscale can enter the human body at the same time, we were interested in determining the types of their combined action (oral administration) on lipid peroxidation, antioxidant protection and circulating immune complexes in experimental animals, as the problem of global water pollution of various reservoirs with these chemicals is currently very relevant.

The aim of the work was to determine the effect of the combined action of nanoparticles of stearates and lead on the state of lipid peroxidation, antioxidant defense, and the level of circulating immune complexes in experimental rats.

Material and methods

The experiments were performed on four groups of Wistar white rats, weighing 150-200 g, with 7 animals in each group. The animals were on the generally accepted diet of the vivarium in the same conditions (t° 22±2°C, light/dark cycle: 12/12 hours) and differed only in the quality of drinking water. Water was taken from the Ternopil city (Ukraine) water supply system, which is fed from the alluvial horizon, located at a depth of 28-32 m. The chemical composition of hydrocarbonate-calcium class water meets the requirements of the state sanitary rules and regulations of Ukraine No. 2.2.4-171-10 (Hygienic requirements for drinking water intended for human consumption). The water was dechlorinated and enriched with sodium and potassium stearates [14].

The first (control) and second groups of animals consumed dechlorinated water from the city water supply. The third and fourth groups of animals consumed water according to the content of sodium stearates and potassium stearate at a dose of 1/250 LD_{50}. After 40 days of use of these waters, animals of the second, third and fourth groups were orally administered lead nanoparticles at a dose of 1/250 of LD_{50}. Three days later, the animals were removed from the experiment by bloodletting under thiopental anesthesia in accordance with the rules of bioethics.

The nanoparticles of lead used in the experiment were manufactured by the Nanomaterials and Nanotechnologies Company (Ukraine) by gas-phase synthesis by evaporation of the metal at a controlled temperature in an atmosphere of inert gas and low pressure with subsequent vapor condensation. According to the quality certificate, the nanoparticles had the form of a homogeneous transparent liquid, a light dark color, were odorless; the pH of the solution was 2.5-7.2 units, the size was 20-70 nm, the lead concentration was 1500.00 mg/dm^3, the density was 1.00015 g/cm^3. The products met the requirements of the state standard.

To determine the types of combined action of nanoparticles and stearates, divisional and additive variants of calculating the coefficient of combined action on the indicators of the central tendency, variability and probability estimates, according to M. Y. Antomonov, were used [15]. The essence of the divisional method is to calculate the coefficient of combined action (D) as the ratio of the effect of the combined action to the average sum of the effects under the separate influence of factors:

$$D = z_{\Sigma} / \hat{z} \qquad (1)$$

where:

z_{Σ} - response rate of the biosystem with combined action;

$\hat{z}$ is the average value of the response of biosystems for all factors.

The unit with the corresponding confidence interval ($1 \pm t_{\alpha,v} \cdot S(D)$) acts as a mathematical criterion for estimating the type of effect. The effect is considered antagonistic if the value of the coefficient D is less than the lower limit of the confidence interval of the mathematical criterion. If the value of D is greater than the upper limit, it is believed that the effect of potentiation has been observed. In the intermediate case, the action of factors is considered independently.

When using the additive method (R), the difference is calculated:

$$R = z_{\Sigma} - \hat{z} \qquad (2)$$

In the additive approach, the characteristic of the combined action is compared with zero:

If $R < - t_{\alpha,v} \cdot S(R)$, then the action of factors is antagonistic,

If $R > - t_{\alpha,v} \cdot S(R)$, then the effect of potentiating factors,

If $t_{\alpha,v} \cdot S(D) \geq D \geq t_{\alpha,v} \cdot S(D)$, then the action of factors must be considered independent at the selected level of significance.

The level of indicators of lipid peroxidation (malonaldehyde (MDA) and diene conjugates (DC)), antioxidant protection (superoxide dismutase (SOD), catalase, ceruloplasmin) and the level of circulating immune complexes (CIC) in blood and homogenates of internal organs were determined using photoelectrocolorimetry. In particular, in organ homogenates and blood serum, the following indicators were determined: MDA, DC, SOD, catalase, and only in blood serum – ceruloplasmin, CIC.

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 (Protocol No. 14 of October 2, 2020) did not find any violations of moral and ethical norms during this study.

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

Shapiro-Wilk test (the number of subjects was less than 50). Changes at $p<0.05$ were considered statistically significant.

Results

The additive effect of these chemicals and potentiation on the change in lipid peroxidation parameters were observed under the action of stearates and lead nanoparticles (Table 1). In particular, changes in MDA in the liver, kidneys and serum showed an additive effect and potentiation to the same extent. However, changes in DC in serum were observed to potentiate the effect of these substances (except potassium stearate and lead nanoparticles), and in the liver and kidneys, there was an additive effect.

Table 1. Types of combined action of stearates and lead nanoparticles by changes in lipid peroxidation in serum, liver, and kidneys in animals

Indicator	Organs	Calculation option	Sodium stearate + lead nanoparticles	Potassium stearate + lead nanoparticles
MDA	Serum	R	Potentiation	Potentiation
		D	Additive action	Potentiation
	Liver	R	Additive action-Potentiation	Additive action
		D	Additive action	Additive action – Potentiation
	Kidneys	R	Potentiation	Potentiation
		D	Potentiation	Potentiation
DC	Serum	R	Potentiation	Additive action
		D	Potentiation	Potentiation
	Liver	R	Additive action	Additive action
		D	Additive action	Additive action
	Kidneys	R	Additive action	Additive action
		D	Additive action	Additive action

Notes: D is divisional version of the calculation; R is an additive calculation option.

The potentiating and additive effect of these chemicals was observed by changing the parameters of the antioxidant system (Table 2). As can be seen from this table, lead nanoparticles and sodium and potassium stearates had a potentiating effect on indicators of catalase and ceruloplasmin in blood serum. Independent action of nanoparticles lead and stearates show catalase in the kidneys, superoxide dismutase (SOD) in serum and liver. Antagonism was observed in the change of superoxide dismutase in the kidneys.

Table 2. Types of combined action of stearates and lead nanoparticles by changes in the antioxidant system in serum, liver, and kidneys in animals

Indicator	Organs	Calculation option	Sodium stearate + lead nanoparticles	Potassium stearate + lead nanoparticles
Catalase	Serum	R	Potentiation	Independent action
		D	Potentiation	Potentiation
	Liver	R	Independent action	Independent action
		D	Potentiation	Independent action
	Kidneys	R	Independent action	Independent action
		D	Independent action	Independent action
SOD	Serum	R	Additive action	Additive action
		D	Additive action	Antagonism – Additive action
	Liver	R	Additive action – Potentiation	Additive action
		D	Additive action	Additive action
	Kidneys	R	Antagonism	Additive action
		D	Antagonism	Antagonism
Ceruloplasmin	Serum	R	Potentiation	Potentiation
		D	Potentiation	Potentiation

Notes: D is divisional version of the calculation; R is an additive calculation option.

The combined effect of sodium stearate and lead nanoparticles, potassium stearate and lead nanoparticles on the content of CIC in animals was characterized by antagonistic action (Table 3), which indicates the counteraction of these chemicals. In this study, all the obtained results are statistically significant.

Table 3. Types of combined action of stearates and lead nanoparticles by changes in CIC in the blood of animals

Indicator	Calculation option	Sodium stearate + lead nanoparticles	Potassium stearate + lead nanoparticles
CIC	R	Antagonism	Antagonism
	D	Antagonism	Antagonism

Notes: D is divisional version of the calculation; R is an additive calculation option.

Discussion

It is common knowledge that lead and its compounds are a priority toxicant. In addition, nowadays it is impossible not to pay attention to new technologies that are developing very rapidly. In all branches of the economy and in medicine, nanotechnology is taking up more and more space [16]. Surface-active substances, such as potassium and sodium stearates, are widely used in the production of detergents, shampoos, hair dyes, hand creams, shaving foams; as an additive to toothpastes and cosmetic creams, they are one of the main components of solid and liquid soap [17]. Despite the wide use of potassium and sodium stearates, there is little data on studying their sanitary-hygienic and toxicological properties.

It is known that the combined action of harmful substances is a simultaneous or sequential effect on the body of several poisons in the same route of entry. A person is often exposed to two or more harmful substances at the same time [18]. Information about the safety and potential risk of nanomaterials is vital both for the health of an adult and for a developing organism. The question of possible antagonism or synergism of nanosized metals, such as microelements, remains undetermined today.

There are several types of combined action of harmful substances [19]:

1. additive action – the effect of substances in combination is summed; the total effect of the mixture is equal to the sum of the effects of the active components;
2. potentiated action – amplification of the effect, one substance enhances the action of another, i.e. affects more than the summation;
3. antagonism – the effect of the combined action is less than expected with a simple summation; one substance weakens the action of another;
4. independent action – the combined effect does not differ from the isolated action of each poison; the effect of the most toxic substance prevails.

A significant increase in the content of lead in the environment (including in surface water) is associated with the burning of coal, the use of tetraethyl lead as an anti-detonator in motor fuel, and the removal of lead into water bodies with wastewater from ore-concentration factories of some metallurgical plants, chemical factories, mines, etc [20].

It is known that the lead content in the locations of enterprises for the processing of spent lead batteries and the production of lead batteries, the background concentration of lead exceeds the MPC in air by more than 1.3 times, in rivers by 2.0 times, and in soils by 4-10 times.

It is also known that a significant amount of stearates enters open reservoirs and water that is later used for water supply to the population. Therefore, the study of the possible adverse effect of stearates on the body of warm-blooded animals, especially in combination with nanoscale lead, will remain an urgent problem of the future for more than one decade.

According to the results of our research, we established that the combined action of sodium stearate and lead nanoparticles, potassium stearate and lead nanoparticles, according to most indicators of lipid peroxidation and antioxidant protection, which were studied, had the character of independent action and potentiation. The antagonistic effect was observed only in changes in SOD in the kidneys and in changes in the CIC index in the blood. The combined effect of sodium stearate and lead acetate, potassium stearate and lead acetate, according to most of the LOP and AOS indicators that were studied, mainly had the character of independent action. Potentiation was manifested by a change in the content of MDA and DC in the blood serum and the content of MDA in the kidneys and by the content of SOD in the target organs of the liver and kidneys and ceruloplasmin in the blood serum. The combined effect of these substances on the content of CIC in animals was characterized by an antagonistic effect. Our research results are consistent with other research data [21,22].

Conclusions

This research showed that the combined effect of lead nanoparticles and stearates was potentiating according to most indicators of lipid peroxidation and the antioxidant system. That is, these pollutants enhanced the effect of each other. However, the antagonistic effect was observed only at the change of SOD in the kidneys and CIC in the blood serum of experimental rats.

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