ANTINOCYCEPTIVE ACTIVITY OF THE COLLOID SOLUTION OF NANOSILVER IN EXPERIMENT

Alexander Nefodov, Ph.D., Associate Professor, Sergey Dronov, Ph.D., Associate Professor, Elena Nefodova, MD, Professor, Vera Shatornaya, Doctor of Biological Sciences, Professor, Natalia Onul, MD, Assistant Professor, Igor Zadesenets, Lecturer of Clinical Anatomy, Anatomy and Operative Surgery, SE "Dnipropetrovsk Medical Academy of the Ministry of Health of Ukraine"

Annotation. In modern science, there is a promising application of nanotechnology in various fields of applied medicine. The interest to the development of products based on nanostructured silver is growing, due to the pharmacological properties that the metal shows at the nanoscale level. The work carried out to study the effect of nanosilver colloid solution on the performance of the antinociceptive activity in models of electrodermal stimulation of rat tail root and "acetic acid writhing". On the model of electrodermal stimulation of rat tail root can suggest a mild analgesic component in a colloidal solution of nanosilver in comparison with the drug - referent that is diclofenac sodium. On the model of "acetic acid writhing" Nanocolloids of silver has a fairly pronounced anti-nociceptive properties comparable to the classical non-opioid analgesic diclofenac sodium. Due to the lack of available data on pain mechanisms nanosilver possible further, more detailed study of the antinociceptive activity in other models of pain in order to identify the alleged mechanisms of anesthesia, as well as expanding the definition of the pharmacological and pharmacodynamic properties. Keywords: nanosilver, pain, analgesics, analgesia.

Relevance. The most common and actual problem is pain - the so-called subjective sensation, close to the feeling of suffering, which is formed in the central nervous system most often during damage to body tissues. A lot of works have been devoted to the pathogenesis and treatment of pain and pain syndromes. One of the first concepts of pain as a phenomenon proposed by Frey at the end of the 19th century was that there are special afferent painful nerve endings (receptors) in the body and ways in which superstrong irritation is transmitted to the brain. In 1965, R. Melzack and P. Wall proposed the theory of "gateway control" of pain, according to which in the spinal cord a special control mechanism regulates the flow of impulses from the periphery to the overlying areas that already manage with nociceptive perceptions. Axons of afferent nociceptive fibers terminate in the columns of the posterior horn of the spinal cord. Here they are in contact with the transfer neurons of the spinotalamic pathway, through which painful impulses reach the posterior nuclei of the thalamus, and then the somatosensory field of the cerebral cortex. The cells of the second segment of the posterior horn form a gelatinous substance (substantia gelatinosa - SG). According to the theory of "gate control" (Wall, Melzack: Gate control theory, 1965), the short

interneurons that make up SG regulate pain impulses from peripheral afferent fibers to the thalamus. The activity of SG-interneurons is subject to modulating influences. They are activated by descending inhibitory neurons or non-nociceptive afferent impulses (for example, by impulses of tactile sensitivity) and inhibited by afferent nociceptive C-fibers. A similar system of "gate control" exists in the thalamus. The results of numerous observations and studies made it possible to form an idea of the existence in the body of an antinociceptive system that suppresses the perception of pain. Structures related to this system include some areas of central gray matter, tegmentum of pons, amygdala, hippocampus, cerebellar nuclei, reticular formation. The existence of an antinociceptive system suggests that its damage can be accompanied by the appearance of pain [1]. Depending on the leading etiopathogenetic

mechanism, the following types of pain are distinguished: 1. nociceptive (somatogenic), associated with tissue damage: a. visceral, b. somatic (parietal); 2. neuropathic (neurogenic), caused by primary dysfunction or damage to the structures of the nervous system; 3. Reflex (reflected, radiating); 4. psychogenic, arising in disorders of the psyche. Among the aforementioned forms of pain, visceral is most often noted. This pain, devoid of localization, characterized by duration, blurriness and very weak degree of regulation, accumulates the main negative nociceptive features of perception in individual and social aspects. These pains occur when the visceral peritoneum is irritated or the capsules of the internal organs are stretched: the liver, spleen, kidney, bladder, or spasm / sudden stretching of any part of the gastrointestinal tract, urinary system. Pains of this type have the nature of attacks and are manifested by so-called colic [2]. According to the step-by-step algorithm for choosing drugs for eliminating of chronic abdominal pain, depending on its intensity and the leading mechanism of development (Fig. 1), exactly analgesics and antispasmodic are the drugs that hold the leading position in the treatment of visceral pain of varying intensity, especially their combined administration. In modern science, using of nanotechnology in various branches of practical medicine seems promising, but they must not only pass all the barriers of experimental and clinical research, in-depth study of pharmacodynamic and pharmacokinetic features, but also take a leading place in modern medicine [4, 5, 6]. The interest in the development of preparations based on nanostructured silver is constantly growing, because of the pharmacological properties that this metal exhibits at the nanoscale level [7, 8].

Fig. 1 the step-by-step algorithm for choosing drugs for eliminating of chronic abdominal pain, depending on its intensity and the leading mechanism of development

Nanoparticles begin to be used for scientific research in the field of biophysics, molecular biology, proteomics, genetics, in particular, for the creation of biomarkers. Magnetic nanoparticles bearing antibodies and DNA fragments have the ability to amplify the signal from many small biomolecules of living structures. This will help diagnose the disease in the early stages and achieve a greater therapeutic effect. In oncology, for the detection of specific tumor markers applies immunological analysis with using stable nanoshells or gold nanowires that change their color during the interaction of the ligand with quantum particles coupled to specific antibodies. In order to increase the efficiency, contrast agents are added to nanoparticles, which, due to their size, surface area and its stability, allow to accumulate contrast exactly where it is necessary for the diagnosis of the pathological process. In addition, nanoparticles themselves can be visualized by various methods: magnetic resonance, ultrasound, fluorescence, nuclear and computed tomography. Gold nanoparticles act as contrast agents. Due to electrostatic and hydrophobic interactions with these particles, any antibody can be attached. At the moment of the interaction of the antibody with the antigen, the nanoparticle changes its color, which is registered with the help of special devices. Nanoparticles can form complexes with products of metabolism of the body, drugs, increasing the solubility of the latter, stabilizing them, as a result the drugs are better absorbed by the cells of the body. Clinical efficacy of pharmacological agents developed by classical methods is often limited to pharmacodynamic and / or pharmacokinetic deficiencies: low efficacy, lack of selectivity, drug resistance at the target organ level, low solubility or bioavailability of the drug, rapid excretion of the drug, etc. Often an insurmountable barrier to drugs on the way to target organs is histohematic barriers (hematoencephalic, hematophthalamic, etc.). However, the biggest problem is the side effects of drugs that significantly restrict the use of some of them, such as cytostatics.

Target delivery systems for medicines should eliminate all the above-mentioned disadvantages, which will significantly increase the effectiveness of drugs. Most of these nanoparticles correspond to these requirements, because due to their small size, such structures easily pass through natural barriers and even membranes of individual cells. In addition, nanoparticles can encapsulate, or bind molecules, which increase solubility, stability and absorption of drugs. The possibility to transfer many substances by nanoparticles was discovered. It includes: DNA, proteins, compounds with a small molecular weight, and in this regard, are best proved by liposomes and polymer nanoparticles, since such structures are subject to biodegradation and are not capable of cumulating. However, for the practical implementation of this idea, further research is needed to closely monitor the delivery of nanosystems to a specific target organ and release of the drug from the system. Promising is the use of nanotechnology in the development of vaccines. Nanoparticle-based vaccines will deliver appropriate antigens directly to dendritic cells, thereby minimizing potential risks. In the scientific literature of the present time, the properties and effects of nanoparticles of silver, copper, aluminum, fullerenes, nanotubes, etc. on the body are quite widely presented. In some studies, toxic and therapeutic doses of nanoproducts have been experimentally determined, but the size of nanoparticles has a significant influence on the toxic effect of the product. It is interesting that the researchers determined different ways of penetration of nanomaterials into the body. For example, in experiments on rats, the toxicity of nanosilver was determined by inhalation administration in different doses. The nanoparticles of silver penetrate to blood initially by means of axonal transport into the olfactory bulb of the brain, which in the future leads to accumulation of substance in the liver of the experimental animals. The results of the study revealed the permeability of nanoparticles not only on the olfactory tract, but also through the blood-brain barrier. This determined the high stability of silver nanoparticles and their ability to sustain toxic effects for a long time. The purpose of our work is to study the effect of a colloidal solution of a nanosilver on the antinociceptive activity at various stimuli. The choice was stopped on this new substance as it is promising as new pharmacological properties and combinations. Materials and methods. In the experimental models, colloidal nanosilver solutions were used, which were purchased according to the original technique at the Institute of Biocolloidal Chemistry. F. D. Ovcharenko National Academy of Sciences of Ukraine (Director: Doctor of Chemistry, Professor Ulberg ZR). Silver nanoparticles (aqueous colloidal solution): the starting materials from which the nanoparticle preparation was synthesized: silver nitrate, potassium carbonate, tannin, water. Form of nanoparticles: spherical. The outgoing concentration of silver nanoparticles is 800 μg / ml by the metal. The size of silver nanoparticles according to laser-correlation spectrometry: (ZAve) 29.9 ± 0.6 nm. The experiments were performed on 120 white nonlinear rats of both sexes weighing 140-270 g, 50 white non-linear mice of both sexes weighing 18-22 g [9, 10, 11]. Animal studies were conducted in accordance with the Methodological

Recommendations of the State Pharmacological Center MoH Ukraine (2001), the "General ethical principles of animal experiments" adopted by the First National Congress on Bioethics (Kiev, 2001), the requirements of the European Convention for the Protection of Laboratory Animals (Strasbourg, 1986), "A provision on the use of animals in biomedical research" [9]. To evaluate the antinociceptive activity, we used the method of electrodermal stimulation of the tail of the rat, while steel needle (0.5 mm in diameter) with a fixed interelectrode distance (10 mm) were inserted under the skin distally 1 cm from

the root of the tail. The pain sensitivity was assessed after 2-3 minutes (the animal calmed down), 30, 60, 90 and 120 minutes after the appearance of a voice reaction (squeak, vocalization) in response to the gradually increasing electrical stimulation from the ESL1 electrostimulator. The duration of each stimulation did not exceed 1 sec [12, 13, 14]. Preparations or saline (control group) were administered once intraperitoneally. For our studies, we used the model of "acetic acidic writhing", which is classical in studying the effect of drugs on visceral pain. The technique is based on the introduction of a 0.6% acetic acid solution intraperitoneally to white mice, which causes the ejection of the pro-pain substances (histamine, serotonin, leukotrienes and prostaglandins), which in turn is manifested by voluntary abdominal l contractions, hinding the limbs and arching of the back [10, 15]. The comparative drug served as a classic non-narcotic analgesic diclofenac sodium at doses of 5 and 10 mg / kg. The experimental data were processed using the STATISTICA 6.1 licensed program, StatPlus 2006 programs, OriginPro 7.5 (OriginLab Corporation, USA), Microsoft Excel 2003. The mathematical processing included calculations of the arithmetic average values (M), their errors (± m). Authentication of the intergroup differences from the experimental data was carried out using the Student's t-test, the Wilcoxon Rank-Sum test, the Mann-Whitney criterion and the ANOVA method [16, 17, 18, 19]. Differences were considered statistically significant at a level of $p < 0.05$. Before applying the parametric criteria, we tested the hypothesis of the normal distribution law for random variables (according to the Kolmogorov-Smirnov criteria with the Lillifors and Schopiro-Wilk amendment). Results and discussion. The results of our experimental studies showed that on the model of electrodermal stimulation of the tail root of rats, the nanosilver colloid (3.5 mg / kg) showed an analgesic effect as early as 30 minutes (22.2%), after which its activity increased and showed its maximum at the 60th minute (52.0%, $p < 0.05$) compared with the baseline background. Subsequently, the pain threshold was gradually restored (at the 90th minute, 18.7%). The drug of comparisson - diclofenac sodium (10 mg / kg) confirmed its pronounced antinociceptive activity. As early as the 30th minute, analgesia was 50.2% ($p < 0.05$) with an analgesia peak at half an hour (82.3%, $p < 0.05$) compared to the baseline background. The obtained experimental data on the "acetic acidic writhing" model showed that the colloidal solution of the nanosilver was sufficiently pronounced to reduce the number of voluntary contractions of the abdominal muscles both in comparison with the control

and the reference drug diclofenac sodium . Thus, the analgesic activity of the colloid was 82.9%, while the comparison drug diclofenac sodium - 86.4% (10 mg / kg) and 78.5% (5 mg / kg) compared to the control group of animals. Conclusions. Thus, on the basis of the foregoing, the following conclusions can be done: 1. on the model of electrodermal stimulation of the tail of the rat, it is possible to assume the presence of a moderate analgesic component at the colloidal solution of the nanosilver in comparison with the reference drug diclofenac sodium; 2. On the model of "acetic acidic writhing" silver nanocolloid has quite pronounced antinociceptive properties comparable to the classical non-opioid analgesic diclofenac sodium; Prospects for further research. Due to the lack of available data on the analgesic mechanisms of the nanosilver, it is possible further study of the antinociceptive activity on other pain models in order to identify the supposed mechanisms of anesthesia, as well as expand the concept of pharmacodynamic and pharmacotherapeutic properties. Conflict of interest. Absent. References:

1. Petrov O.V., Lihvantsev V.V., Subbotin V.V. (1998) Information concept of pain. International Medical Journal, 2, 198-210. 2. Kukushkin M.L., Reshetnyak V.K., Vorobeychik Ya.M. (1994) Neurogenic pain syndromes and their pathogenetic therapy. Anaesthesiology and Reanimatology, 4, 3641. 3. Baranskaya

E.K. (2003) Abdominal pain: clinical approach to the patient and treatment algorithm. Place of spasmolytic therapy in the treatment of abdominal pain. Gastroenterology, 14 (109), 45-49. 4. Chekman I.S. (2011) [Nanopharmacology]. Kyyiv, Zadruha, 424. 5. Nefodov A., Nefodova E., Chatornaya V., Zadesenets I., Onul N., Halperin A., Sharavara L. (2017) Features impact nanosilver colloidal solution on the morphological and biochemical parameters in rats. Modern Science - Moderní věda. 3, 140-147. 6. Chekman I.S., Ul'berh Z.R., Malanchuk V.O., Horchakova N.O., Zupanets' I.A. (2012) [Nanoscience, nanobiology, nanofarmation Kyyiv, Polihraf plyus, 328. 7. Nefedov A.A. (2013) Study of the effect of colloidal nanosilver solution on nociception in the experiment. Materials of the Eastern European Congress on Pain. 35. 8. Nef'odov O.O., Hayduk T.A., Smandych V.S., Hrynevych O.O., Zhyvytsya Ye.V. (2011) Influence of colloidal solution of nanosilver on visceral pain in experiment. Abstracts of the National Scientific and Practical Conference with International Participation "Pain Syndromes in Medical Practice". 14. 9. Kozhem'yakin Yu. M., Khromov O. S., Filonenko M. A., Sayretdinova H. A. (2002). [Scientific - practical advice on keeping laboratory animals and to and from them]. Kiev, 155.

10. Stefanov O. V. [Preclinical studies of drugs: guidelines]. Kiev, Avitsena, 527. 11. Zapadnyuk I. P., Zapadnyuk V. I., Zahariya E. A., Zapadnyuk B. V. (1983). [Laboratory animals. Breeding, content, use in experiment]. Kiev, Vyisshaya shkola. General publishing, 383. 12. D'Amour F.E., Smith D.L. (1941) A method for determining loss of pain sensation. J.Pharmacol. and Exp.Ther. 72, 74-79. 13. Mihaylov V.A., Ignatov Yu.D. [Pain syndrome]. Leningrad, Meditsina,336. 14. Eddy N.B., Leimbach D. (1953) Synthetic Analgesics: Dithienylbutenyl and dithienylbutylamines. Y. Pharmacol. Exp. Ther. 107, 385-389. 15. Vasilev Yu.N., DmitrievA .V., Ignatov Yu.D. (1978) Psycho- and neurophysiological analysis of the antinociceptive effect and its role in the pharmacological correction of the emotional stress of pain genesis. Proceedings of the Institute "Neuropharmacological aspects of emotional stress and drug dependence". 27-38. 16. Lakin G.F. (1990). [Biometrics]. Moscow, Vyisshaya shkola, 293. 17. Lapach S.N., Chubenko A.V., Babich P.N. (2001) [Statistical methods in biomedical research using EXCEL]. Kiev, Morion, 408. 18. Mintser O.P., Ugarov B.N., Vlasov V.V. (1982) [Methods of processing medical information]. Kiev, Vyshcha shkola, 160. 19. Strelkov R.B. (1986) Statistical tables for accelerated quantification of the pharmacological effect. Pharmacology and toxicology, 4, 100-104.