ORIGINAL ARTICLE

CONTENTS

The effect of succinic acid in liposomal emulsion on the humoral component of the immune system of rats

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ABSTRACT

Aim: To investigate the effect of succinic acid on the humoral component of the immune system in rats.

Materials and Methods: The study was conducted on two groups of mature non-linear white rats (males) of similar weight (200-270 g, aged 6-8 months), with 5 animals in each group. The control group was fed a standard diet with free access to water throughout the experiment. Rats in the experimental group were subcutaneously injected with a 0,1% solution of succinic acid in a liposomal emulsion at a dose of 20 cm^3 for five days. The state of the humoral component of the immune system was assessed by measuring serum immunoglobulins A, M, and G using solid-phase enzyme-linked immunosorbent assay. Circulating immune complexes were determined in a 40% solution of polyethylene glycol.

Results: Under the influence of succinic acid in liposomal emulsion the content of class A immunoglobulins increased by 44,0% ($p<0.01$) compared to rats in the control group. The activation of class A immunoglobulin synthesis points to the provision of local immunity of the mucous membranes in the rat's body. The content of class M immunoglobulins increased by 61,0% ($p<0.001$) compared to the control group rats. This high activity of class M immunoglobulins indicates their rapid activation in the body of rats in response to primary contact with succinic acid in liposomal emulsion. The content of class G immunoglobulins increased by 36,0% ($p<0.05$) compared to the control group rats. No clinical deviations from physiological norms were observed in the rats after the use of succinic acid in liposomal emulsion. After the use of succinic acid in liposomal emulsion in the experimental group of rats, the concentration of CICs increased by 15,0% ($p<0.05$) compared to the control group rats. In our case, the increase in CIC levels is not correlated with clinical manifestations but is a consequence of increased levels of class M and G immunoglobulins.

Conclusions: The succinic acid in liposomal emulsion activates the production of class A, M, G immunoglobulins, circulating immune complexes, it prevents the development of secondary immunodeficiency and has a positive impact on the humoral branch of the immune system in rats.

KEY WORDS: succinic acid, immune system, humoral immunity, rats, liposomal emulsion, immunoglobulins

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INTRODUCTION

The immune system serves to protect the body from various genetically foreign factors [1]. Humoral factors (interferons, interleukins, chemokines), as well as components of the immune system such as the complement system, lysozyme, and immunoglobulins, play a significant role in the immune response [2, 3].

Disruption of the innate or adaptive immunity components can lead to the development of immunodeficiency conditions [4, 5]. Primary immunodeficiencies are caused by congenital dysfunctions of the immune system, typically of genetic etiology, while secondary immunodeficiencies result from other factors, often of exogenous origin, leading to immune system exhaustion [6, 7].

Currently, there has been limited progress in the use of immunotropic drugs [8].

A significant portion of the pharmaceutical market is occupied by synthetic immunotropic drugs, which are often associated with numerous adverse side effects. An alternative to these is herbal-based medicines [9, 10].

In immunotherapy, herbal drugs and medications are used to stimulate the synthesis of interferons (e.g., Aloe arborescens, Kalanchoe pinnata), influence the complement system (e.g., basil, mountain arnica) [11], and increase lysozyme levels in the body (e.g., anise, eucalyptus) [12].

According to the State Register of Medicines in Ukraine, as of 2024, approximately 1,020 immunomodulatory drugs are registered under the L category according to the ATC classification, with 10% being domestic products and the remaining 90% being of foreign origin.

Succinic acid and its salts, or succinates, are important intermediates in metabolism, possessing protective properties for the body. Succinic acid is present in all living organisms in certain quantities as it is produced in the mitochondria [13].

Research data [14] indicates that succinic acid is harmless to the body and has several beneficial properties: it positively influences oxygen absorption by cells, exhibits anti-stress and adaptogenic effects, and more. It is known that succinic acid not only has detoxifying properties but also stimulates cellular respiration, enhances the body's resistance, and improves the absorption of nutrients due to its chelating properties [15].

Another important property of succinic acid is its ability to influence immune defense processes in infectious diseases [16, 17]. The immunostimulating properties of succinic acid form the basis of some medications. It should be noted that succinic acid is non-toxic to the body, does not accumulate, and can therefore be used over prolonged periods [18].

Medications based on succinic acid activate aerobic oxidation processes, have antioxidant and antihypoxic effects, and can reduce free radicals and restore cellular immunity [19]. Succinic acid-based medications have also been shown to be effective in the treatment and prevention of influenza, forming a stable immunity [20].

One of the modern approaches in medicine and pharmacy is the development of drug delivery systems that enhance the bioavailability and efficacy of therapeutic substances. One such approach is the creation of liposomal drugs [21].

There are few studies on the effect of succinic acid on the humoral component of the immune system in rats, making the development and search for effective prevention schemes of immunodeficiency conditions relevant.

AIM

To investigate the effect of succinic acid on the humoral component of the immune system in rats.

MATERIALS AND METHODS

In the first stage of the study, a liposomal emulsion with succinic acid was prepared using an ultrasonic disperser УЗДН-А at a working frequency of 22 kHz at the Ternopil Research Station of the National Academy of Agrarian Sciences of Ukraine. To enhance the strength of the liposomal emulsion membranes, up to 40% cholesterol was added to the initial lipid mixture. The internal aqueous volume of the liposomes contained a 0.1% solution of succinic acid [22].

In the second stage, the effect of succinic acid in the liposomal emulsion on the humoral component of the immune system in rats was studied.

The study was conducted on two groups of mature non-linear white rats (males) of similar weight (200-270 g, aged 6-8 months), with 5 animals in each group. The rats were kept under identical conditions at the vivarium of I. Horbachevsky Ternopil National Medical University. The control group (Group I) was fed a standard diet with free access to water throughout the experiment. Rats in the experimental group (Group II) were subcutaneously injected with a 0,

1% solution of succinic acid in a liposomal emulsion at a dose of 20 cm^3 for five days.

Clinical research was conducted using a typical parallel group model. Rats were randomly selected for the groups to achieve statistically significant results.

On the fourth day after the last injection of succinic acid in the liposomal emulsion, the rats were decapitated under thiopental anesthesia. Blood was collected aseptically into two test tubes: one with heparin for morphological studies and another without heparin for biochemical studies.

The state of the humoral component of the immune system was assessed by measuring serum immunoglobulins A, M, and G using solid-phase enzyme-linked immunosorbent assay (ELISA) with an "eBioscience Inc." reagent kit and a "StatFax" analyzer [23]. Circulating immune complexes were determined in a 40% solution of polyethylene glycol [24].

The care of rats and all experiments involving animals were conducted in accordance with the provisions of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 2005) and the Law of Ukraine "On the Protection of Animals from Cruelty".

Statistical processing of the study results was performed using Microsoft Excel 2003. The significance was assessed using Student's t-test, and the results were considered statistically significant at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$.

RESULTS

To determine the effect of succinic acid in liposomal emulsion on the state of the humoral link of the immune system of rats, the content of immunoglobulins A, M, G, and CIC in the blood of rats before and after the use of the drug was investigated.

The obtained data indicate that under the influence of succinic acid in liposomal emulsion the content of class A immunoglobulins increased by 44,0% ($p<0.01$) compared to rats in the control group (Table 1). The obtained data

Table 1. The content of immunoglobulins A, M, G and CICs in the blood of rats after the use of succinic acid in a liposomal emulsion (M±m, n = 5)

Groups	Indicators			
	Immunoglobulins, A, g/l	Immunoglobulins, M, g/l	Immunoglobulins, G, g/l	Circulating immune complexes, con. un.
Control	0,36 ±0,02	0,90 ± 0,06	2,24 ± 0,07	32,50 ± 1,45
Experiment	0,52 ± 0,06**	1,45 ± 0,09***	3,04 ± 0,05*	37,52 ± 1,53*

Note. * – $p < 0,05$; ** – $p < 0,01$; *** – $p < 0,001$ compare with control group.

give grounds for asserting that under the influence of succinic acid in liposomal emulsion there is activation of plasma cells located under the mucous membranes, which facilitated the production of class A immunoglobulins. The activation of class A immunoglobulin synthesis under the influence of succinic acid points to the provision of local immunity of the mucous membranes in the rat's body, as serum immunoglobulin class A protects the respiratory, urinary tracts, and gastrointestinal tract from various infections. These antibodies prevent bacteria from attaching to epithelial cells, thus inhibiting their adhesion and preventing bacterial damage to cells. Consequently, succinic acid in liposomal emulsion, along with nonspecific immune factors, ensures the protection of mucous membranes from viruses and microorganisms. Thus, succinic acid in liposomal emulsion has a positive effect on the formation of immunity in mucosa in rats.

From the data presented in Table 1, it can be seen that after the application of succinic acid in liposomal emulsion in the experimental group of rats, the content of class M immunoglobulins increased by 61,0% ($p<0.001$) compared to the control group rats. This high activity of class M immunoglobulins indicates their rapid activation in the body of rats in response to primary contact with succinic acid in liposomal emulsion.

Given that class M immunoglobulin is part of the antigen-specific receptor of B-lymphocytes, and IgM is found in monomer form on the cell membrane with an additional hydrophobic domain, succinic acid in liposomal emulsion promoted the activation of B-lymphocytes that secrete pentameric IgM, later switching to IgG or other immunoglobulin classes. IgM is the first barrier against infection. Although it has low specificity (affinity) for antigens, its pentameric structure allows it to bind five antigen molecules simultaneously, resulting in high avidity binding. Furthermore, due to its oligomeric nature, IgM easily causes the agglutination of microbial cells, aiding their destruction by macrophages.

Therefore, it can be assumed that succinic acid in liposomal emulsion activates the production of class M immunoglobulins, prevents the development of secondary (acquired) immunodeficiency, and has a positive impact on the humoral branch of the immune system in rats.

The data in Table 1 also show that after the application of succinic acid in liposomal emulsion in the experimental group of rats, the content of class G immunoglobulins increased by 36,0% ($p<0.05$) compared to the control group rats. No clinical deviations from physiological norms were observed in the rats after the use of succinic acid in liposomal emulsion. Therefore, the increase in the content of class G immunoglobulins in the rats' bodies after using succinic acid in liposomal emulsion indicates the induction of antibody-dependent cell-mediated cytotoxicity and the formation of long-term humoral immunity.

Circulating immune complexes (CICs) form as a result of the immune response to foreign antigens. The immune system reacts to antigens that enter from outside (during viral, bacterial, parasitic infections, etc.), as well as to autoantigens formed during physiological or pathological processes in the body. These complexes are usually quickly removed by the phagocytic system. The mechanisms of their elimination are linked to a complex of cellular, biochemical, and enzymatic mechanisms, including the activation of the complement system.

After the use of succinic acid in liposomal emulsion in the experimental group of rats, the concentration of CICs increased by 15,0% ($p<0.05$) compared to the control group rats. In our case, the increase in CIC levels is not correlated with clinical manifestations but is a consequence of increased levels of class M and G immunoglobulins.

DISCUSSION

Blood tests are an important diagnostic method, a way to control treatment effectiveness, and a marker of recovery. Biochemical blood analysis allows for an assessment of the functioning of internal organs and systems. Some biochemical blood parameters are markers that determine the magnitude of the inflammatory process.

Immunity is one of the elements of homeostasis in the body. The immune system ensures immune responses that contribute to the preservation of genetic homeostasis.

After the application of succinic acid in liposomal emulsion in the experimental group of rats, the con-

tent of class A immunoglobulins increased by 44,0% ($p<0.01$) compared to the control group rats. The obtained data allow us to state that under the influence of succinic acid in liposomal emulsion, there was an activation of plasma cells in the rats' bodies aimed at producing class A immunoglobulins, which ensure the local protection of mucous membranes.

Thus, succinic acid in liposomal emulsion positively affects the formation of immunity in the mucous membranes of rats. Our data supplement research [6], which reports that succinic acid prevents the loss of IgA neutralizing activity during the massive entry of antigens, including superantigens, through the mucous membranes, and reports [15] regarding increased resistance of the body due to succinic acid exposure.

Up to 10,0% of the total amount of immunoglobulins is accounted for by class M immunoglobulins. These immunoglobulins are effective in agglutination and opsonization reactions and bind complement well. After the application of succinic acid in liposomal emulsion in the experimental group of rats, the content of class M immunoglobulins increased by 61,0% ($p<0.001$) compared to the control group rats.

This high activity of class M immunoglobulins indicates their rapid activation in the rats' bodies in response to the primary contact with succinic acid in liposomal emulsion. Therefore, it can be assumed that succinic acid in liposomal emulsion activates the production of class M immunoglobulins, prevents the development of secondary (acquired) immunodeficiency, and has a positive effect on the humoral branch of the immune system in rats. This expands upon reports [6] that reduced immune insufficiency underlies immunodeficiency and that the use of succinic acid in liposomal emulsion positively affects the body's metabolic processes, complementing reports [16] on its use as a regulator of metabolic processes.

From 70,0 % to 85,0 % of the total amount of immunoglobulins is accounted for by class G immunoglobulins. It should be noted that these immunoglobulins are found in the body's tissue fluids, and during the immune response, they appear in the serum following class M immunoglobulins.

After the application of succinic acid in liposomal emulsion in the experimental group of rats, the content of class G immunoglobulins increased by 36,0% ($p<0.05$) compared to the control group rats. No clinical deviations from physiological norms were observed in the rats after using succinic acid in liposomal emulsion. Therefore, the increase in the content of class G immunoglobulins in the rats' bodies after using succinic acid in liposomal emulsion indicates the formation of long-term humoral immunity. Our data expand on reports [8] about the immunomodulatory properties of succinic acid.

Circulating immune complexes form in response to foreign antigens. The immune system reacts to antigens that come from outside (during viral, bacterial, parasitic infections, etc.), as well as to autoantigens formed during physiological or pathological processes.

After the application of succinic acid in liposomal emulsion in the experimental group of rats, the concentration of circulating immune complexes increased by 15,0% ($p<0.05$) compared to the control group rats.

The increase in CIC concentration by 15,0% is a result of decreased tolerance to autoantigens and impaired elimination processes. Succinic acid in liposomal emulsion dissolves and circulates in the rats' bloodstream, leading to its slight accumulation on the membranes of small vessels and tissues, which subsequently causes an increase in the concentration of circulating immune complexes. The elevated CIC level is not specific to any particular disease and does not indicate immune complex pathology or tissue damage.

CONCLUSIONS

Succinic acid in liposomal emulsion positively influences the humoral branch of the immune system in rats, as evidenced by the increase in immunoglobulin class A by 44,0% ($p<0.01$), immunoglobulin class M by 61,0% ($p<0.001$), immunoglobulin class G by 36,0% ($p<0.05$), and circulating immune complexes by 15,0% ($p<0.05$).

REFERENCES

1. Konstantynenko LA, Abramovych AM. Otsinka imunnoho statusu orhanizmu [Assessment of the body's immune status]. Aktual'ni problemy laboratornoyi diahnostyky: materialy I rehional'noyi naukovo-prakt.2018;40-48. (Ukrainian)
2. Nicholson L. The immune system. Essays Biochem. 2016;60(3):275-301. doi: 10.1042/EBC20160017. DOI
3. Harahulia H, Severyn R, Momot A, Zhunko I. Imunna systema ptytsi ta ssavtsiv: porivnialna kharakterystyka [Immune system of poultry and mammals: comparative characteristics]. Ahrarnyy visnyk Prychornomor'ya. 2022;5:41-58. (Ukrainian)
4. Khodakov O et al. Imunodefitsytni stany v klinichnii praktytsi : navch. posib. [Immunodeficiency states in clinical practice: especially possible.]. Dnipro: DZ «Dnipropetr. med. akad. 2019, p.228. (Ukrainian)
5. Bihuniak T, Charnosh S, Bihuniak K. Patohenetychni aspekty pervynnykh imunodefitsytiv [Pathogenetic aspects of primary immunodeficiencies]. Monografia pokonferencyjna. 2019, pp.43-48. (Ukrainian)

6. Maltsev DV. Klasyfikatsiia, klinichni proiavy, diahnostyka i likuvannia pervynnykh minornykh imunodefitsytiv liudyny [Classification, clinical manifestations, diagnosis and treatment of primary minor human immunodeficiencies]. Klinichna imunolohiya. Alerholohiya. Infektolohiya. 2021;1(130):20-28. (Ukrainian)
7. Vishchur O, Hutyi D, Khariv I. Imunnyi status, sposoby otsinky i metody korektsii u teliat rannoho viku: monohrafiia [Immune status, evaluation methods and methods of correction in young calves: monograph]. Lviv: SPOLOM. 2015, p.183. (Ukrainian)
8. Khalieieva OL. Farmakolohichna kharakterystyka kompleksnykh imunotropnykh fitopreparativ, predstavlenykh na ukrainskomu farmatsevtychnomu rynku [Pharmacological characteristics of complex immunotropic herbal preparations presented on the Ukrainian pharmaceutical market]. Materials of the First International science and practice conf. 2017;1:319-323. (Ukrainian)
9. Tykhonov OI, Koval VM, Shpychak OS. Analiz farmatsevtychnoho rynku likarskykh zasobiv imunomoduliuvalnoi dii v Ukraini [Analysis of the pharmaceutical market of immunomodulatory drugs in Ukraine]. Sotsial'na farmatsiya v okhoroni zdorov'ya. 2018;4(4):76–84. (Ukrainian)
10. Chekman I, Syrovaia A, Makarov V. Yantar, yantarna kyslota, suktsynaty (monohrafiia) [Amber, succinic acid, succinates (monograph).] Kh.: TOV «Planetaprynt». 2017, p.107. (Ukrainian)
11. Najafi H, Yarijani ZM, Changizi-Ashtiyani S et al. Protective effect of Malva sylvestris L. extract in ischemia-reperfusion induced acute kidney and remote liver injury. PLoS One. 2017;12(11):e0188270. doi: 10.1371/journal.pone.0188270. DOI
12. Bletskan M, Svystak V. Osoblyvosti zastosuvannia fitoterapii pry ozhyrinni [Peculiarities of the use of phytotherapy in obesity Ukraine]. Zdorov'ya natsiyi. 2018;2(49):5-8. (Ukrainian)
13. Palchevska TA, Ziuzia LO, Kozachok HS et al. Burshtynova kyslota ta yii katalitychnyi syntez. Fizyko-orhanichna khimiia, farmakolohiia ta farmatsevtychna tekhnolohiia biolohichno aktyvnykh rechovyn [Succinic acid and its catalytic synthesis. Physico-organic chemistry, pharmacology and pharmaceutical technology of biologically active substance]. Zbirnyk naukovykh prats' / za zah.red. A. F. Popov. Kyiv. 2018, pp.149-154. (Ukrainian)
14. Kryvonosova OV, Ziuzia LO. Burshtynova kyslota ta yii pokhidni, sposoby otrymannia, zastosuvannia u farmatsii. Naukovi rozrobky molodi na suchasnomu etapi [Succinic acid and its derivatives, methods of production, use in pharmacy. Scientific developments of youth at the current stage]. Promyslova farmatsiya. Kyyivs'kyy natsional'nyy universytet tekhnolohiy ta dyzaynu. 2017, pp. 554-555. (Ukrainian)
15. Yefimov VH, Rakytianskyi VM. Pokaznyky klitynnoho imunitetu porosiat na doroshchuvanni za vplyvu humatu natriiu, burshtynovoi kysloty i mikroelementiv [Indicators of cellular immunity of piglets under the influence of sodium humate, succinic acid and trace elements]. Naukovyy visnyk L'vivs'koho natsional'noho universytetu veterynarnoyi medytsyny ta biotekhnolohiy imeni S. Z. Hzhyts'koho. 2015;17(3):32-37. (Ukrainian)
16. Pidborska RV. Burshtynova kyslota yak rehuliator obminnykh protsesiv [Succinic acid as a regulator of metabolic processes]. Tezy dopovidey Mizhnarodnoyi naukovo-prakt. 2015, p.11. (Ukrainian)
17. Labenska IB. Burshtynova kyslota–potentsiinyi farmakofor pry modeliuvanni novykh biorehuliatoriv na osnovi azotovmisnykh heterotsykliv [Succinic acid is a potential pharmacophore in the modeling of new bioregulators based on nitrogen-containing heterocycles]. Farmakolohiya ta likars'ka toksykolohiya. 2016;2:.3-13. (Ukrainian)
18. Fedoniuk L, Stravskyy Y, Khavtur V, Sachuk R. Prevention of subinvolution of the uterus of cows using acidum succinicum. ScienceRise: Biological Science. 2021;2(27):49-52. doi: 10.15587/2519-8025.2021.235936. DOI
19. Lysenko YuS. Zastosuvannia burshtynovoi kysloty dlia oderzhannia likarskykh preparative [The use of succinic acid for the production of medicinal products]. Avtoreferat mahisters'koyi roboty. Kyiv. 2020, p.9. (Ukrainian)
20. Garofalo S, Cocozza G, Porzia A. Natural killer cells modulate motor neuron-immune cell cross talk in models of amyotrophic lateral sclerosis. Nat Commun. 2020;11(1):1773. doi: 10.1038/s41467-020-15644-8. DOI
21. Lutsiv YeR, Stravskyi YaS. Liposomy: kharakterystyka ta vykorystannia u medytsyni [Liposomes: characteristics and use in medicine]. Modern engineering and innovative technologies. 2023;26(4):53-65. (Ukrainian)
22. Stadnichenko O, Krasnopolskyi Y, Yarnykh T. Development of pilot technology for liposomal irinotecan production. Current issues in pharmacy and medicine: science and practice. 2018;17(1): 37-41. doi:10.14739/2409-2932.2018.2.133184. DOI
23. Vlizlo VV, Fedoruk RS, Ratych IB et al. Laboratorni metody doslidzhen u biolohii, tvarynnytstvi ta veterynarnii medytsyni. [Laboratory research methods in biology, animal husbandry and veterinary medicine]. Lviv: SPOLOM. 2012, p.764. (Ukrainian)
24. Levchenko VI, Vlizlo VV, Kondrakhin IP. Veterynarna klinichna biokhimiia [Veterinary clinical biochemistry]. Bila Tserkva. 2002, p.399. (Ukrainian)

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

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