Comparative Evaluation Of The Effect Of Pyrimidine Mapic, Derivatives Of Maleopimaric Acid In Combination With Anylocaine And Polylophacin On The Cell- And Non-Cell-Mediated Immune Response

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Abstract: *Of all the components of medicinal plant materials all over the world, essential oils deserve the greatest interest, including resin acids available in the bark of many wild and cultivated trees. Considering that the key element in the mechanism of action of resin acids and their derivatives is the anti-inflammatory effect, the research results reflected in this*

article, confirm their indirect effect, contributing to an increase in the IL-2 cytolytic function of T-killers and NK cells, which increase production of perforin and IFN-γ by these cells, activating monocytes and macrophages, which increase the synthesis and secretion of TNF-α, IL-1β, IL-6, IL-8, granulocyte colony-stimulating factor (G-CSF), GM-CSF. Such a biological effect of the components of herbal medicine provides the most optimal pharmacological effect in the abovementioned pathological conditions with the least possible negative side effects, which is confirmed by randomized clinical studies in world practice.

The information presented in the article reveals further prospects for studying the biological activity of resin acids, which provide a combined pharmacological effect in a number of pathological conditions in the organism of animals, both for individual use and in combination with more specific medicinal substances. At the same time, the low cost of the proposed triterpenoids undoubtedly provides their advantages in the pharmaceutical market for veterinary medicine, and in productive animal husbandry it opens up prospects for use as a means of enhancing nonspecific immunity.

Keywords: *pharmacology, immunology, triterpenoids, maleopimaric acid derivatives, screening.*

INTRODUCTION

In modern conditions of a wide variety of drugs used in the treatment and prevention of animal diseases, rationalization of pharmacotherapy is of particular importance, involving the use of multi-directional drugs, including compositions of several active substances with a synergistic effect against each other (Liu et al., 2014; Yue et al., 2017).

In the development of such tools, the greatest interest of world practice is given to the direction of modifying the chemical structure of components of wild-growing and cultivated medicinal plants (Alakurtti et al., 2006; Chen et al., 2016). A distinctive feature of these medicinal substances over synthetic ones is the possibility of obtaining a number of positive effects and advantages, including: expansion of the spectrum of pharmacological action (Sun et al., 2017); relatively low toxicity parameters to the organism of warm-blooded animals; high biological activity and bioavailability with targeted pathways (Kazakova et al., 2010); good tolerability and low probability of adverse reactions of the body (Kazakova et al., 2010); renewability of sources of raw materials for production (Alakurtti et al., 2006); environmental friendliness of production and safety of use during long courses of treatment (Kazakova et al., 2010; Liu et al., 2014) and many others.

Considering the features of the essential oils contained in the bark of many trees as sources for the design of new biologically active compounds, it is necessary to note clear advantages in pyrimidines, as well as adducts of abietic, quino- and maleopimaric acids (Alakurtti et al., 2006; Drąg-Zalesińska et al., 2017; Kazakova et al., 2010). Many researchers indicate the presence of a wide range of already identified and experimentally confirmed pharmacological properties of these compounds: antimicrobial, anti-viral, anti-inflammatory (Laavola et al., 2016) and anti-ulcer (Yadav & Chandra, 2017); Ishikawa et al., 2017, antirhematoid (Thoonsen, 2017), antioxidant (Laavola et al., 2016) anti-lysing (Bertone, 2005), growth-promoting properties (Kazakova, et al., 2013) and others.

There is evidence (Lin A.J. et al., 1992) that the enantiomerically pure abietic acid serves as a ligand for the γ-receptors of the cell nucleus, activating proliferation by peroxis (PPAR-γ), including in activated macrophages, which may cause anti-inflammatory activity (Lin et al., 1992). The same molecular mechanism underlies the ability of abietic acid to regulate carbohydrate and lipid metabolism, which allows its use for the treatment of atherosclerosis (Lee et al., 2017; Pennini et al., 2017).

In their publication the authors Olivares N., Rodriguez Y., Zatarain-Barron Z.L., et al. (2017) point out the manifestation of anti-tuberculosis and the pronounced manifestation of adjuvant properties by derivatives of terpenoids that reduce the probability of the emergence of microflora resistant to antibiotics during prolonged treatment (Olivares et al., 2017).

The opinion about the possibility of obtaining an anticancer effect with the use of abietic acid and its derivatives is ambiguous. So Miura N., Matsumoto Y., Miyairi S. et al. (1999) revealed the ability of derivatives of terpenoids to inhibit the promoter activity of 12-O-tetra-decanoylforbol-13-acetate in a

two-stage carcinogenesis model induced in the skin of mice by dimethylbenzenthracene. In addition, the possible cytotoxic activity of abietic acid derivatives with respect to cervical cancer cells *HeLa* and hepatocellular carcinoma *HepG*$_2$ has been described (Miura et al., 1999). In contrast, Japanese researchers Cho S.H., Gottlieb K., and Santhanam U. (1994) established the ability of abietic acid to inhibit protein-mediated P-gp and MRP-2 transporters of transmembrane efflux substances from cells, which can cause multidrug resistance of malignant cells to antineoplastic drugs, in connection with which there is an opinion about its ability to reduce the resistance of tumor cells to chemotherapy (Cho, Gottlieb & Santhanam, 1996). But it is worth noting that both of these examples are not a direct contradiction to each other, but only prove the possibility of plastic change of a set of biological properties by modifying the chemical structure of terpenoids, which was later confirmed by studies of Hirova K., Takahashi T., Miura N. et al. (2002). They studied the cytotoxic, antimycotic and antiviral activities of the derivatives of abietic and dehydroabietic acids and found that the introduction of an aldehyde group into the *C*18 position increases the severity of their biological activity, and the introduction of a hydroxyl or carboxyl group – decreases it (Hiroya et al., 2002). They also established that the compounds of the terpene series with the peroxide fragment increase the antitumor activity and provide the manifestation of the antimalarial property.

Taking into account the promising development of new drugs based on terpenoids, we have developed a scheme for the synthesis of 21 new, previously unexplored derivatives of resin acids (abietic acid, its ozonate, quinopimaric acid and 5 of its derivatives; maleopimaric acid and 13 of its linear amides and amides with amino acid residues). Analysis of the probable types of biological activity of resin derivatives and their severity, carried out using authorized access to the international computer system *PASS C & T* (*Prediction of Activity Spectra for Substances: Complex & Training*), established the most promising compounds (MEK - monomethyl ester of ketotetracarboxylic acid; OmeMPA - methyl ester of maleopimaric acid), taking into account the possible quantitative and qualitative set of types of expected pharmacological activity. Experimental studies have confirmed the pronounced anti-ulcer, anti-inflammatory, hepatoprotective activity and antioxidant properties, as well as high efficacy in the treatment of diseases of the respiratory, digestive and urinary systems in agricultural and non-productive animals, including when combined with Anilokaine and Polifloxacin antibiotic as part of complex pharmacotherapy.

It should be noted that all our early studies were carried out on animals with pathologies accompanied by secondary immunodeficiency states. Our further research was to uncover possible additional pharmacological properties of the studied compounds, including those that influence the cell-mediated and non-cell-mediated immune response. Moreover, when conducting a computerized *PASS C & T* analysis, we found that the probability of immunostimulating activity when using MEK is 60.3 ± 2.3% (which is ~ 2 times higher than that of abietic acid), while the probability of immunosuppressive activity was only 10.1 ± 0.9%, and their ratio is 6.0: 1.0.

In turn, the goal of our research was to experimentally confirm the presence of immunostimulating, immunomodulating or immunosuppressive properties. It reveals the prospects for further targeted use of derivatives of maleopimaric acid as any disruption of the natural immune response on the part of the body leads to chronic primary disease and further complications in the treatment of animals.

In the course of research, the following tasks were set:

- study of the effect of MEK, OmeMPA and their compositions with anilokaine (A) and polyfloxacin (P) on the functional ability of T-effectors in the delayed-type hypersensitivity reaction (DTHR) provoked by dinitrofluorobenzene dissolved in acetone (non-cell-mediated immune response), also by immunization by intravenous injection of sheep erythrocytes in a sensitizing dose (cell-mediated immune reaction) when compared with the activity of the base maleopimaric acid (MA) and the closest analogue - MAHP (2-methyl-4-amino-6-hydroxy-pyrimidine);

- study of the effect of the abovementioned compounds on the dynamics of neutrophil phagocytic activity (as the most aggressive phagocytic forms of leukocytes), as well as phagocytic activity of peritoneal macrophages.

MATERIALS AND METHODS

The object of the study was new derivatives of maleopimaric acid (MEK and OmeMPA), the biological activity of which was compared with the activity of the base maleopimaric acid (MA), the closest analogue of MAHP, as well as with anilokaine (A), polyfloxacin (P), including their combinations: MAHP+A+

Enrofloxacin (1:2:0.2); MAHP+A+P (1:2:0.2); MEK+A+P (1:2:0.2); OmeMPA+A+P (1:2:0.2); MEK+A+P (1:2:0.2).

As laboratory animals, calibrated laboratory mice (M=19...21 g) were used, which were kept in the same conditions of the vivarium meeting the requirements of "Sanitary rules for the design, equipment and maintenance of experimental biological clinics (vivariums)" #1045-73, and also State Standard P 53434-2009 "Principles of Good Practice", in accordance with international standards *GLP*. The diet of mice consisted of balanced by nutrition, vitamins and minerals granulated feed "ProKorm", the corresponding to State Stadard P 50258-92 "Complete feed for laboratory animals."

When studying the effect of the studied compounds on non-cell mediated immunity in white outbred mice, a sensitization reaction was caused by applying 0.05 ml of a 1% solution of dinitrofluorobenzene in acetone on the right side. 10 days after the sensitization, the mice were divided into 25 groups (23 experimental and 2 control) with 7 animals each. Subsequently, mice on both sides of the right ear were applied with 0.05 ml of 0.1% dinitrofluorobenzene solution (resolving dose). Simultaneously the mice of experimental groups received intragastrically the studied compounds (MAHP, Anilokain (A), Polifloxacin (P), maleopimaric acid (MPA), maleopimaric acid methyl ester (OmeMPA), methyl ester of tetracarboxycarbonic acid (META) and their combinations in various doses. Diclofenac was intragastrically injected to animals of the first control group (positive control) at a recommended dose of 8 mg / kg, animals of the second control group (negative control) were intragastrically injected with placebo (0.9% sodium chloride solution). When studying the effect of the studied compounds on cell-mediated immunity, sensitization of 25 groups of mice was carried out by intravenous injection of a sensitizing dose of sheep erythrocytes (2×10^6 cells), and after 10 days a resolution dose of sheep erythrocytes (2×10 cells) was injected subplantably into the right paw of mice, with intragastric injection of the same compounds as in the sensitization experiments with dinitrofluorobenzene. 24 hours after the use of resolving doses of sensitizers and the use of the studied compounds in the doses studied, the extent of change in the mass of the affected ear (in the experiments with dinitrofluorobenzene) and the change in the weight of the paws of mice (in experiments with sheep erythrocytes) in comparison with the control ones were determined.

The study of the possible modulation of the MEK + A + P composition (1: 2: 0.2) of antibody genesis was performed on white non-inbred mice weighing 19-22 g. Mice were immunized with intraperitoneal injection of an optimal dose (2×10^8) of sheep erythrocytes. Simultaneously with the immunization, the experimental animals received the composition of MEK + A + P (1: 2: 0.2) intragastrically in doses of 25 mg / kg, 50 mg / kg and 100 mg / kg for 7 days; the composition of the comparison: MAHP + A + E (1: 2: 0.2) and MAHP + A + P (1: 2: 0.2) in doses of 25 mg / kg; animals of the control group received intragastrically placebo (0.9% sodium chloride solution). After 7 days, mice of the experimental and control groups were euthanized by the cervical method under ether anesthesia, the spleen weights removed from them were homogenized in RPMI 1640 medium and used to determine the anti-body forming cells in the reaction with the compliment. Serum was isolated from blood collected from mice and the level of hemagglutinins was determined.

The research to determine the effect of the studied compounds on the phagocytic activity of neutrophils in peripheral blood and peritoneal macrophages was performed on 60 mice of the first generation hybrids (F_1=$C_{57}Bl$♂ × CBA♀), divided into 12 groups. The animals received the investigated compositions MAHP + A + E (1: 2: 0.2), MAHP + A + P (1: 2: 0.2) and MEK + A + P (1: 2: 0.2) in the studied doses at a single use for 24 hours before the start of research and sevenfold use within 7 days. The functional activity of peritoneal macrophages was assessed by the ability to reduce nitrosynium tetrazolium and pharmacans to diformazan under the action of the products of the NADP-oxidase reaction upon activation of macrophages (“Breathing Explosion”). The number of peritoneal macrophages carrying *Fc*–receptors was estimated by rosetting with sheep erythrocytes (EA-rosetting cell). Determination of neutrophil phagocytic activity was carried out with a suspension of killed microbial bodies of *Staphylococcus aureus* (strain 209).

Statistical analysis obtained in the course of research results was carried out using statistical software SAS (statistical analysis system [SAS]). In this case, we used the method of univariate analysis of variance (ANOVA) for unrelated samples, including the determination of mean values (M), mean errors (m), standard deviations of the sample (δ), the degree of confidence by Student.

RESULTS

Screening studies have shown that the compounds under study, along with the comparator drug (diclofenac), do not cause the formation of a delayed-type hypersensitivity reaction in experimental animals. At the same time, data from table 1 show that MAHP, Anilokain and Polifloxacin, with a non-cell-mediated reaction, have a lower activity in terms of lowering the percentage increase in ear edema than with therapeutic use of diclofenac, whereas when formulating a cell-mediated reaction, diclofenac activity is lower.

Table 1. The effect of MAHP, Anilokain, Polifloxacin and maleopimaric acid derivatives and their combinations on the delayed-type hypersensitivity reaction provoked by dinitrofluorobenzene and sheep erythrocytes

Compound name	Dose, mg/kg	Quantity of mice in group	% increase edema (dinitroftorobenzene)	% increase edema (sheep erythrocytes)
MAHP	25	7	44.70±0.52#	32.12±0.64*#
Anilokain	50	7	38.15±0.67#	35.18±0.55*#
Polifloxacin	5	7	40.54±0.65#	38.62±0.53*#
MAHP+A+E (1:2:0.2)	25	7	36.15±0.61#	25.14±0.67*#
MAHP+A+P (1:2:0.2)	25	7	32.12±0.67*#	23.60±0.56*#
MA	25	7	32.14±0.62*#	29.45±0.56*#
	50	7	32.45±0.60*#	28.18±0.57*#
	100	7	32.70±0.66*#	28.64±0.67*#
*Ome*MPA	25	7	33.00±0.58*#	24.00±0.54*#
	50	7	32.17±0.62*#	25.78±0.62*#
	100	7	34.72±0.55*#	25.48±0.62*#
MEK	25	7	31.22±0.67*#	25.09±0.66*#
	50	7	31.17±0.59*#	25.80±0.56*#
	100	7	35.24±0.52#	25.20±0.63*#
MPA+A+P	25	7	29.76±0.69*#	24.89±0.62*#
(1:2:0.2)	50	7	28.24±0.61*#	25.61±0.66*#
	100	7	29.11±0.67*#	24.65±0.56*#
*Ome*MPA+A+P	25	7	31.17±0.68*#	24.11±0.53*#
(1:2:0.2)	50	7	32.19±0.55*#	23.32±0.57*#
	100	7	33.10±0.67*#	23.98±0.54*#
MEK+A+P	25	7	26.64±0.58*#	20.61±0.63*#
(1:2:0,2)	50	7	26.64±0.68*#	21.08±0.61*#
	100	7	28.18±0.62*#	20.94±0.61*#
Diclofenac	8	7	36.84±0.56#	44.86±0.60#
Control (placebo)	–	7	48.63±0.60	57.29±0.67

Note: * - P <0.05 compared with diclofenac; # - P <0.02 compared with control (placebo)

This indicates a more pronounced effect of these compounds on the cell and non-cell-mediated immune response, compared with the humoral. An increase in the inhibitory effect on the development of inflammatory edema was also noted with the replacement of enrofloxacin in the MAHP + A + E composition with polyfloxacin. The prerequisites for the replacement of MAHP in the composition of MAHP + A + P by triterpenoids, derivatives of maleopimaric acid, were the fact that MEK, OmMPA and MEK, when used individually at doses of 25-50 mg / kg, cause more pronounced inhibition of inflammatory edema. The formed, new, MEK + A + P, OmeMPA + A + P and MEK + A + P compositions justified the alleged increase in the inhibition of edema with the highest effect in the therapeutic single use of the composition MEK + A + P (1: 2: 0.2) in a dose of 25 mg / kg. At the same time, the inhibition of edema of the ears was expressed: by 10.2% stronger compared with diclofenac (26.64 ± 0.58% versus 36.84 ± 0.56%) and 21.99% compared with the intact control (26.64 ± 0.58% versus 48.63 ± 0.60%) in the reaction of the formation of non-cell hypersensitivity under the action of dinitrofluorobenzene: 24.25% more efficient than diclofenac (20.61 ± 0.63% against 44.86 ± 0.60%) and 36.68% compared with placebo (20.61 ± 0.63% versus 57.29 ± 0.67%) in the formation of cell-mediated hypersensitivity under the influence of sheep erythrocytes in a sensitizing dose. The presence of a positive effect in both series of experiments also indicates the possibility of simultaneous stimulation of non-cell and cell-mediated humoral immune responses under the influence of the MEK + A + P composition (1: 2: 0.2).

The marked suppression of the delayed-type hypersensitivity reaction in the administration of MAHP, anilokaine, polyfloxacin, MA, OmeMPA, MEK and their compositions seems to be associated with the presence of pronounced anti-inflammatory properties.

In order to confirm the data obtained during screening studies, we conducted four more series of experiments to identify the possible development of a delayed-type hypersensitivity reaction to the MEK + A + P composition (1: 2: 0.2) with prophylactic (prescribed for 5 days before sensitization) and curative (prescribed for 5 days after sensitization) use of high-breed mice of C_{57}Bl and CBA lines sensitized by sheep erythrocytes (Table 2).

Table 2. The effect of the composition of MEK + A + P (1: 2: 0.2) on the formation of delayed-type hypersensitivity reaction to sheep erythrocytes

Compound name	Dose, mg/kg	Quantity of mice in group	% increase edema (dinitroftorobenzene)	% increase edema (sheep erythrocytes)
$C_{57}Bl$				
MAHP+A+E (1:2:0.2)	25	15	16.24±0.50*#	13.26±0.66*#
MAHP+A+P (1:2:0.2)	25	15	15.32±0.68*#	12.74±0.57*#
MEK+A+P (1:2:0.2)	25	15	14.29±0.65*#	11.24±0.61*#
	50	15	12.46±0.61*#	10.80±0.53*#
	100	15	12.51±0.68*#	10.80±0.67*#
Diclofenac	8	15	16.46±0.62*#	14.68±0.58*#
Control (placebo)	–	15	22.20±0.59*#	18.34±0.50*#
CBA				
MAHP+A+E (1:2:0.2)	25	15	13.28±0.61*#	15.59±0.68*#
MAHP+A+P (1:2:0.2)	25	15	12.66±0.53*#	14.34±0.69*#

MEK+A+P	25	15	11.94±0.67*#	12.67±0.54*#
(1:2:0.2)	50	15	10.76±0.58*#	11.08±0.68*#
	100	15	11.00±0.50*#	11.46±0.57*#
Diclofenac	8	15	15.24±0.70#	16.29±0.68#
Control (placebo)	–	15	19.2±0.61	21.13±0.62

Note: * - *P <0.05* compared with diclofenac; # - *P <0.02* compared with placebo.

When comparing the data obtained during screening studies and the data given in Table 2, we can observe direct proportional and reliable dependences of effective suppression of inflammatory edema of the legs of highly inbred C_{57}Bl and CBA mice in both prophylactic and therapeutic use of MEK + A + P composition (1: 2). : 0,2) at a dose of 25 mg / kg.

In order to confirm the effectiveness of MEK + A + P composition (1: 2: 0.2) on the model of the humoral immune response, we carried out a qualitative assessment of changes in the B-link by determining the number of antibody-forming cells (AFC) in the spleen, based on the ability of anti-erythrocyte bodies, secreted by antibody-forming cells of immunized animals to mutate in the presence of complement of sheep erythrocytes placed in a monolayer (Table 3).

Table 3. The effect of the composition of the MEK + A + P (1: 2: 0.2) on the formation of AFC

Compound name	Dose, mg/kg	Quantity of mice in group	Number of AFC×10^6 spleenocytes	The titer of hemagglutinin of blood serum
MAHP+A+E	25	15	1599.42±44.93*	3.39±0.37#
(1:2:0.2)				
MAHP+A+P	25	15	1701.84±72.11*	3.73±0.42#
(1:2:0.2)				
MEK+A+P	25	15	2008.73±43.15*	5.76±0.18#
(1:2:0.2)	50	15	2177.34±64.91*	5.33±0.31#
	100	15	2268.12±59.37*	5.64±0.24#
Control (placebo)	–	15	534.56±12.71	1.80±0.06#

Note: * – *P*<0,002; # – *P*<0,05

As can be seen from table 3, the use of the composition MEK+ A + P (1: 2: 0.2) at a dose of 25 mg / kg leads to a significant increase in the formation of AFC mice in the spleen (by 1,474.17 AFC more than in the control group) and it shows a 1.26 times greater efficiency compared with the MAPP + A + E composition (1: 2: 0.2) and is 1.18 times more effective than the MAHP + A + P composition (1: 2: 0.2) in the same doses. A similar trend was observed on the side of increasing the serum hemagglutinin titer to the level of 5.76 ± 0.18 when using the MEK + A + P composition (1: 2: 0.2) at a dose of 25 mg / kg against a lower serum hemagglutinin titer the blood of mice treated with the composition MAHP + A + E (1: 2: 0.2) and MAHP + A + P (1: 2: 0.2) in doses of 25 mg / kg, 1.7 and 1.54 times respectively.

Increasing the dose of the MEK + A + P composition (1: 2: 0.2) to 50 mg / kg and 100 mg / kg did not lead to a significant increase in the antibody-forming activity compared with a dose of 25 mg / kg.

When conducting research to identify the effect of the studied compounds on the phagocytic activity of neutrophils of peripheral blood and peritoneal macrophages, it was established that just a day after a single use of the studied compounds, a noticeable increase in the phagocytic activity of macrophages occurs.

Thus, in the group of animals to whom the composition MEK / A + P was prescribed (1: 2: 0.2) at a dose of 25 mg / kg, the color activity indicator (CAI) of macrophages characterized a more pronounced degree of irritation, reflected in an increase in the percentage of pharmasen-positive cells (with the deposition of diformazan in the cytoplasm) during the formulation of the cytochemical reaction with nitro blue tetrazolium. The color index in this case amounted to 0.047 ± 0.004, which is 3.36 times more when compared with the color indicator in the control group, as well as 1.52 and 2.24 times more compared with the same color indicator in groups of animals treated with the MAHP compositions + A + Enrofloxacin (1: 2: 0.2), MAHP + A + P (1: 2: 0.2) in the same doses, respectively (Table 4).

Table 4. Dynamics of changes in the functional activity of peritoneal macrophages to sheep erythrocytes

Compound tested	Number EA- rosetting cells %		Number EA for 100 macrophages		CPA of macrophages	
	once	sevenfold	once	sevenfold	once	sevenfold
MAHP+A+E (1:2:0.2), 25 mg/kg	38.70 ±2.45*	53.00 ±3.43#	147.60 ±17.08&	212.20 ±17.38#	0.021 ±0.003#	0.071 ±0.003#
MAHP+A+P (1:2:0.2), 25 mg/kg	43.40 ±2.32#	59.80 ±3.19#	159.80 ±12.10#	226.00 ±18.09$	0.031 ±0.001#	0.079 ±0.003#
MEK+A+P (1:2:0.2), 25 mg/kg	54.80 ±2.04#	74.30 ±3.2$	259.50 ±20.99#	384.60 ±15.36$	0.047 ±0.003#	0.123 ±0.001$
MEK+A+P (1:2:0.2), 50 mg/kg	56.10 ±2.60#	71.10 ±2.18$	255.80 ±22.46#	363.30 ±12.65$	0.047 ±0.004#	0.126 ±0.002$
MEK+A+P (1:2:0.2), 100 mg/kg	56.50 ±2.32#	72.40 ±2.32$	265.80 ±11.20#	367.70 ±12.28$	0.048 ±0.001#	0.124 ±0.002$
Control (placebo)	30.50 ±1.90	31.00 ±1.33	97.00 ±6.11	99.60 ±3.44	0.014 ±0.004	0.012 ±0.002

Note: * – *P*<0,05; # – *P*<0,02; *$* – *P*<0,005

In addition to increasing the phagocytic activity of peritoneal macrophages with a single prescription of the composition MEK + A + P (1: 2: 0.2) by mouth at a dose of 25 mg / kg, we noted a significant activation of the Fc-receptor apparatus, reflected in an increase in the percentage of EA-rosetting cells by ~ 1.8 times compared with the control (54.80 ± 2, 04% EA- rosetting cells in the experiment against 30.50 ± 1.90% EA- rosetting cells in the control), as well as an increase in the number of sheep erythrocytes (EA) attached to 100 macrophages, ~ 2.67 times compared to the control (259,50 ± 20,99 EA / 100 macrophages in the experiment against 97,00 ± 6,11 EA / 100 macrophages in the control). Whereas, when used once by mouth the compositions MAHP + A + P (1: 2: 0.2) and MAHP + A + E (1: 2: 0.2) in similar doses (25 mg / kg), these indicators were significantly less pronounced: the percentage of EA-rosetting cells was higher compared to the control by ~ 1.42 and ~ 1.27 times, respectively, which is 38% and 53%, respectively, less compared to the percentage of EA- rosetting cells in the group of animals, once receiving the MEK composition + A + P (1: 2: 0.2) at a dose of 25 mg / kg by mouth. The number of EA forming rosets with peritoneal macrophages in groups of mice that received once the composition MAHP + A + P (1: 2: 0.2) and MAHP + A + E (1: 2: 0.2) in doses of 25 mg / kg by mouth it was more when compared with the control in ~ 1.64 and ~ 1.52 times, respectively, which is ~ 1.8 times less when compared with the group of mice that received the composition MEK + A + P (1: 2: 0.2) by mouth in the same dose of 25 mg / kg.

Table 5. Dynamics of changes in the functional activity of peripheral blood neutrophils in killed microbial cells of *Staphylococcus aureus* (strain 209)

Compound tested	Phagocytic activity of neutrofiles, %		Number of phagocytic microb bodies, units		Phagocytic index		Phagocytic number	
	once	sevenfold	once	sevenfold	once o	sevenfold	once	sevenfold
MAHP+A + Enrofloxacin (1:2:0.2), 25 mg/kg	41.70 ±1.57*	60.80 ±1.62#	176.40 ±6.65$	277.10 ±20.5$	4.23 ±0.02*	4.56 ±0.30*	1.76 ±0.07*	2.77 ±0.21#
MAHP+A+P (1:2:0.2), 25 mg/kg	47.80 ±1.62#	64.30 ±1.89#$	208.60 ±17.22$	308.60 ±19.22	4.36 ±0.23*	4.80 ±0.28*	2.09 ±0.17*	3.09 ±0.19#
MEK+A+P (1:2:0.2), 25 mg/kg	59.00 ±3.02#	79.30 ±3.23$	318.70 ±21.46$	451.60 ±24.33	5.40 ±0.10*	5.70 ±0.30*	3.19 ±0.21#	4.52 ±0.24#
MEK+A+P (1:2:0.2), 50 mg/kg	61.30 ±3.23#	79.10 ±3.18$	334.10 ±25.39$	441.30 ±28.14	5.44 ±0.14*	5.58 ±0.33*	3.34 ±0.25#	4.41 ±0.28#
MEK+A+P (1:2:0.2), 100 mg/kg	61.10 ±2.18#	78.60 ±2.07#$	317.90 ±24.95$	438.20 ±28.94	5.20 ±0.37*	5.57 ±0.34*	3.18 ±0.25#	4.38 ±0.29#
Control (placebo)	30.30 ±1.89	30.80 ±2.04	97.30 ±6.58	95.90 ±7.95	3.22 ±0.22	3.12 ±0.23	0.97 ±0.07	0.96 ±0.08

Note: * – *P*<0,05; # – *P*<0,02; *$* – *P*<0,005

Sevenfold use of the studied compounds led to an even greater and, at the same time, a steady increase in both the activity and aggressiveness of peritoneal macrophages and peripheral blood neutrophils, but without going beyond permissible limits. So, at sevenfold use during the 7 days of the prescription of the composition MEK + A + P (1: 2: 0.2) at a dose of 25 mg / kg, the rosetting activity of peritoneal macrophages and neutrophils increased by ~ 1.36 times (from 54.80 ± 2.04% EA- rosetting cells to 74.30 ± 3.23% EA- rosetting cells) and ~ 1.34 times (from 59.00 ± 3.02% to 79.30 ± 3.23%), respectively; the aggressiveness of peritoneal macrophages increased ~ 1.48 times (from 259.50 ± 20.99 EA / 100 macrophages to 384.60 ± 15.36 EA / 100 macrophages), with an increase in the phagocytic index by ~ 9.3% (from 4.74 ± 0.09 to 5.18 ± 0.11), and neutrophil aggressiveness by ~ 42% (from 318.70 ± 21.46 to 451.60 ± 24.33 phagocytosed killed microbial bodies of *Staphylococcus aureus* per 100 neutrophils). A similar trend was noted in relation to all studied compositions.

DISCUSSIONS

When comparing the phagocytic activity of peritoneal macrophages to EA and phagocytic activity of peripheral blood neutrophils to killed microbial bodies *Staphylococcus aureus* (strain 209) - table 5 - it can be noted that the percentage of cells involved in the phagocytosis is distributed approximately in equal proportions for the investigated compositions, but the aggressiveness of blood neutrophils is significantly higher than the aggressiveness of peritoneal macrophages. So, if in the group of animals that received the composition MEK + A + P (1: 2: 0.2) once by mouth at a dose of 25 mg / kg, one peritoneal macrophage on average formed an roset with 4.71 ± 0.09 EA, the phagocytic index of blood neutrophils to the microbial bodies of killed *Staphylococcus aureus* (strain 209) was 5.40 ± 0.10, which is 14% more than in peritoneal macrophages.

Based on the results of the study, it should be noted that increasing the dose of the MEK + A + P composition (1: 2: 0.2) to 50 and 100 mg / kg did not result in significantly more pronounced changes in

the activity of peritoneal macrophages and peripheral blood neutrophils in comparison with the prescription of the composition MEK + A + P (1: 2: 0.2) at a dose of 25 mg / kg either once or repeatedly. A similar conclusion was obtained when studying the processes of synthesis and the characteristics of an imide of modified poly (dimethylsiloxane) with maleopimaric acid as a raw material (Xu et al., 2015).

Other authors studied the effect of maleopimaric acid allyl ester (MPA) on the rheological properties of modifying low density polyethylene with itaconic acid in the presence of free radical initiators. It was found that the addition of ester 84a in an amount of 1 wt. % reaction system reduces vaccination efficiency by only 13% (Bej, Juvchenko & Sokol, 2017))

Derivatives of Mannosylglycerate were also synthesized and a preliminary biological study of their ability to behave as immunostimulating agents was conducted (25). Namely, their toxicity to mononuclear cells of peripheral blood from healthy donors and their ability to enhance phagocytosis of opsonized bacteria by polynuclear neutrophils has been investigated (25).

Several N-aryl maleopimaric acid diimides (3a-3d, 4a-4g) were synthesized and evaluated their topoisomerase I inhibitory activities along with cytotoxicities against NCI, MGC-803, Bel-7404 and Hct-116 cell lines. All the synthesized compounds did not show cytotoxicity against HUVEC cells. In addition, maleopimaric diimides exhibited stronger cytotoxicity and topoisomerase I inhibitory activity compared with maleopimaric acid (Yao et al., 2016).

It could be observed that the curing product of MPA modified vinyl poly(dimethylsiloxane) exhibited the most excellent thermal stability, which should be ascribed to the incorporation of imide heterocycle (Hamciuc et al., 2011).

CONCLUSION

The screening studies have experimentally confirmed the pronounced immunostimulating activity of ketotetracarboxylic acid monomethyl ester and maleopimaric acid methyl ester synthesized by us. Moreover, this type of biological activity turned out to be significantly higher than the maleopimaric acid and the closest analogue, MAHP, used for the synthesis of the base acid. It was determined that the immunostimulating effect, both for prophylactic and therapeutic purposes of the studied compounds, is ensured by simultaneously enhancing cellular and humoral immune responses. In addition, MEK and OmeMPA exhibit a synergistic effect while simultaneously using the fluoroquinolone series (Enrofloxacin, polyfloxacin) with antibiotics and the nonsteroidal anti-inflammatory agent - anilokaine. The possibility of combining the derivatives of maleopimaric acid with anilokaine and fluoroquinolones used in the experiments outlines the great prospects for their use in complex pharmacotherapy for the widespread diseases of the respiratory, digestive, hepatobiliary, urogenital systems, etc. processes, including secondary immunodeficiency. According to the research results, the use of MEK and OmeMPA as individual immunostimulants is not excluded, but it is noted that the highest efficiency can be obtained from a single prescription of the composition MEK + A + P (1: 2: 0.2) at a dose of 25 mg / kg prescrition by mouth with the preventive purpose in case of the threat of the occurrence of immunodeficiency, and sevenfold - with the medical purpose.

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