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Anti-Inflammatory And Immunomodulating Activity of the 1(10) B-Epoxy-5,7α,6β(H)-Guai-3(4),11(13)-Dien-6,12-Olide

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Abstract

BACKGROUND: The article discusses the results of studies of the anti-inflammatory and immunomodulatory activity of the sesquiterpene lactone 1(10)β-epoxy-5,7α,6β(H)-guai-3(4),11(13)-diene-6,12-olide (hereinafter referred to as epoxyguaianolide).

AIM: The article discusses the results of studies of the anti-inflammatory and immunomodulatory activity of the sesquiterpene lactone 1(10)β-epoxy-5,7α,6β(H)-guai-3(4),11(13)-diene-6,12-olide.

MATERIALS AND METHODS: A non-steroidal anti-inflammatory drug diclofenac sodium at a dose of 25 mg/kg (ampoules, 2 mL each, manufactured by Germany) was used as a reference drug in models of acute aseptic inflammation and cotton ball granuloma. On the model of cyclophosphamide immunosuppression, the immunomodulator levamisole at a dose of 3 mg/kg, was used as a reference drug. The immunomodulating effect of the test samples was studied on outbred adult male rats and outbred mice. We calculated the arithmetic mean, the mean square error of the arithmetic mean, the significance of the difference between the mean P_t according to the student's t-test (TTEST), and the significance of the difference in P_f variances according to the Fisher f-test.

RESULTS: The results of the experiments indicate the presence of a pronounced immunomodulatory activity in epoxyguaianolide. When interacting with cells of the immune system, epoxyguaianolide activated the studied parameters in animals with immunosuppression, whereas in intact animals, it did not affect the above parameters. On the model of cyclophosphamide immunosuppression, it was found that epoxyguaianolide has a pronounced immunomodulatory effect, manifested in an increase in the number of T-lymphocytes and a subpopulation of theophylline-resistant T-lymphocytes, an increase in the number of theophylline-sensitive T- and B-lymphocytes, activation of phagocytosis processes, and increased cell migration. This action of epoxyguaianolide eliminates the minimal possibility of hyperactivation of the immune system, which is an important condition for its use as an immunotropic agent.

CONCLUSIONS: The conducted experiments made it possible to recommend epoxyguaianolide for clinical trials as an immunomodulating agent in the treatment of chronic inflammatory diseases accompanied by secondary immunodeficiencies.

Introduction

The search for compounds with pronounced immunobiological properties, but with low cytotoxicity, is a difficult task for immunopharmacological studies.

Sufficiently high immunomodulatory and anti-inflammatory properties of epoxyguaianolide, as well as modern concepts of the pathogenesis of inflammatory and immune processes, indicate its possible complex effect on the inflammatory process. Given the above properties of epoxyguaianolide, it is necessary to further study various methods of treating chronic inflammatory diseases, not only etiologically, but also pathogenetically.

The problem of treating infectious and inflammatory diseases of various organs and systems is extremely relevant [1].

Recently, more and more information has been accumulated about the key role of the immune system in the development of inflammatory processes that form the basis of chronic diseases of the female genital organs [2], [3]. In this regard, the problem of an objective assessment of the immune status and immunocorrection of the identified disorders becomes relevant.

One of the ways to solve the problem is to search for natural immunomodulators with anti-inflammatory action. In this regard, plant sesquiterpene lactones are of interest, most of which have antitumor, antiprotozoal, immunomodulatory, antibacterial, and antiviral effects, which can be the basis for the development of new drugs [2], [4].

For sesquiterpene lactones, a wide spectrum of biological activity has been described, including immunobiological properties that manifest themselves in a strong activation of cytokine secretion [5], [6], [7], [8].

The specific immunostimulatory effect (activation of nitric oxide synthase [NOS], secretion of interferon-gamma, interleukin [IL-2]) of sesquiterpene lactones depends on the dose and structure of compounds that can be used as the basis of anticancer, anti-infective, and immunomodulatory agents [9].

Taking into account the previously established anti-inflammatory and immunomodulatory properties of sesquiterpene lactones 1(10)β-epoxy-5,7α,6β(H)-guai-3(4),11(13)-diene-6,12-olid (hereinafter - epoxyguaianolide) (Figure 1) is of interest to study its effect on inflammation and the immune system.

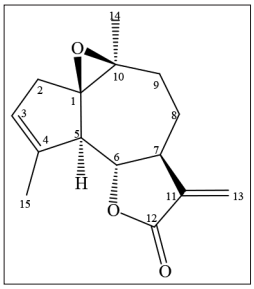

Figure 1: Structural formula of 1(10)β-epoxy-5,7α,6β(H)-guai- 3(4),11(13)-diene-6,12-olid

Epoxyguaianolide (Figure 1) is a colorless crystalline substance of composition $C_{15}H_{18}O_3$, m.p. 100–102°C (hexane), $[\alpha]^{20}_D$ + 45° (c 0.3; chloroform).

A serious problem hindering the use of natural sesquiterpene γ-lactones in medical practice is their insolubility in water. Therefore, we have studied the immunomodulatory activity of epoxyguaianolide (Figure 1) and its water-soluble form, dimethylaminoderivative hydrochloride (Figure 2).

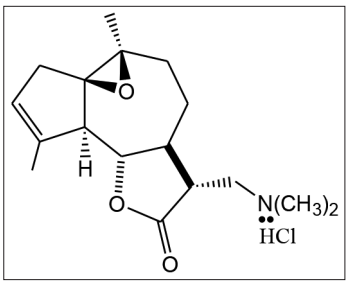

Figure 2: Structural formula of dimethylaminoepoxyguaianolide hydrochloride (B)

Dimethylaminoepoxyguaianolide hydrochloride (Figure 2) is a white crystalline substance of composition $C_{17}H_{26}O_3NCl$, m.p. 203-204°C (ethanol), $[\alpha]^{21}$ + 61,53° (c 0.52; chloroform). It is soluble in ethanol, DMSO, and water. Production of LLP "Karaganda Pharmaceutical Plant."

Materials and Methods of Research

A non-steroidal anti-inflammatory drug diclofenac sodium at a dose of 25 mg/kg was used as a reference drug in models of acute aseptic inflammation and cotton ball granuloma. Diclofenac sodium was used in the form of ready-made aqueous solutions (ampoules, 2 mL each, manufactured by Merckle GmbH for ratiopharm GmbH [Germany]).

On the model of cyclophosphamide immunosuppression, the immunomodulator levamisole (2,3,5,6-tetrahydro-6-phenylimidazo-[2,1-b]-thiazole hydrochloride) at a dose of 3 mg/kg, which is an imidazole derivative, was used as a reference drug [10], [11].

The immunomodulating effect of the test samples was studied on outbred adult male rats and outbred mice. A solution of cyclophosphamide was injected intraperitoneally at a lymphotoxic dose of 50 mg/kg, 1 day before the start of drug administration. Samples (Figures 1 and 2) were administered intragastrically in starch mucus daily for 15 days, Levamisole - every other day orally, for 15 days (8 injections). The control group of animals was injected with starchy mucus in equivolume amounts.

For statistical processing, Microsoft Excel was used. We calculated the arithmetic mean, the mean square error of the arithmetic mean, the significance of the difference between the mean P_t according to the student's t-test (TTEST), and the significance of the difference in P_f variances according to the Fisher f-test.

Results and its Discussion

At the first stage, a comparative analysis of the anti-inflammatory properties of epoxyguaianolide (Figure 1) and its dimethylaminoderivative hydrochloride (Figure 2) was carried out. On the model of acute aseptic inflammation induced by 1% carrageenan, it was noted that epoxyguaianolide (Figure 1) when administered intragastrically in all doses (5–25–50 mg/kg) has a pronounced anti-exudative effect. At the peak of the maximum development of edema (4 h), the degree of inhibition was 40–65%, whereas the anti-inflammatory effect of epoxyguaianolide at doses of 25 and 50 mg/kg significantly exceeded those of the comparison group (diclofenac sodium 25 mg/kg). With intraperitoneal administration, similar dynamics of edema volume were noted, but the degree of inhibition at all doses and time points exceeded by 5–10% the indicators obtained with intragastric administration. Apparently, a less pronounced effect (Figure 1) with enteral administration is associated with its partial destruction in the gastric environment and slower absorption.

Dimethylaminoepoxyguaianolide hydrochloride (Figure 2) in both routes of administration also had a pronounced antiexudative effect, but in terms of the severity of the therapeutic effect, it was slightly inferior to epoxyguaianolide (Figure 1).

It is important to note that epoxy-guaianolide (Figure 1) and hydrochloride derivative (Figure 2) showed activity from the 1st h of the development of carrageenan edema. It is an established fact that when modeling the indicated edema, its development in the first 10–20 min is associated with degranulation of mast cells and the release of inflammatory mediators - histamine and serotonin, within 1–2 h is supported by bradykinin and other kinins accumulating in the focus of inflammation, and starting from the 3rd h - prostaglandins type E. Taking into account the above data, we assumed the effect of the studied lactones on the triggers of inflammation.

This assumption was confirmed in the model of histamine-induced edema. Already by 15 min, a pronounced anti-exudative effect of epoxyguaianolide at doses of 25 and 50 mg/kg was observed - edema inhibition was 50 and 60%, respectively. By 45–90 min, the effect of epoxy-guaianolide increased significantly (66–87% → 89–93%, depending on the dose), and by 120 min, complete inhibition of edema was noted. The values of the hydrochloride derivative up to 45 min were inferior to those obtained in animals treated with epoxy-guaianolide (Figure 1), and from 90 min, the anti-inflammatory properties of both tested substances were comparable. The degree of inhibition of histamine edema by sodium diclofenac throughout the entire period of active observation was 42–46%.

In addition, on the model of formalin edema of the paws of rats, it was found that both test samples have a pronounced anti-exudative activity. Epoxyguaianolide (Figure 1) and dimethylaminoderivative hydrochloride (Figure 2) at a dose of 5 mg/kg after 4 h showed an anti-inflammatory effect comparable to that of diclofenac. With an increase in the dose of epoxyguaianolide (Figure 1), a significant increase in the antiexudative effect was noted. After 24 h, epoxy-guaianolide (Figure 1) in all doses significantly exceeded the anti-inflammatory effect of diclofenac, and complete relief of edema was registered on days 3–4. For hydrochloride (Figure 2) and the reference drug, complete relief of inflammatory edema was observed on days 4–5.

The above experimental data indicate a pronounced dose-dependent effect of epoxy-guaianolide (Figure 1) and dimethylaminoderivative hydrochloride (Figure 2) at the stage of exudation.

The nature of the effect of epoxy-guaianolide (Figure 1) on the proliferation phase was determined by the formation of granulation-fibrous tissue around a cotton ball implanted under the skin of experimental animals under sterile conditions under ether anesthesia. It has been established that epoxyguaianolide (Figure 1) inhibits the formation of granulation-fibrous tissue in the focus of inflammation in all tested doses and is slightly inferior in terms of the severity of the antiproliferative effect to the reference drug diclofenac sodium. At the same time, the severity of the effect of the hydrochloride derivative was 1.2–1.3 times less than that of epoxy-guaianolide.

Currently, the anti-inflammatory properties of sesquiterpene lactones are associated with the inhibition of transcription factors Nuclear factor-kappa B (NF-kB) and NF-AT [12], [13], [14], [15], [16], which accelerate the transcription of inflammatory response genes (pro-inflammatory cytokines, the cyclooxygenase gene, NOS, immunoreceptors, cell adhesion molecules, acute phase proteins, hematopoietic growth factors and their receptors) during viral and bacterial infections and other stress factors in μm concentrations. Thus, inhibition of NF-kB can significantly reduce the inflammatory process. In [17], [18], it is suggested that sesquiterpene lactones inhibit the activation of NF-kB by inhibiting the degradation of factors IkB-α and IkB-β. On the other hand, the sesquiterpene lactone parthenolide (Figure 3) has been shown to inhibit NF-kB, the STAT3 signaling protein, and nuclear factor of activated T -mediated transcription of anti-apoptotic genes. Also, parthenolide (Figure 3) inhibits gene expression induced by IL-6-type cytokines by blocking the phosphorylation of the STAT3 signaling protein on tyrosine 705, which explains its anti-inflammatory activity.

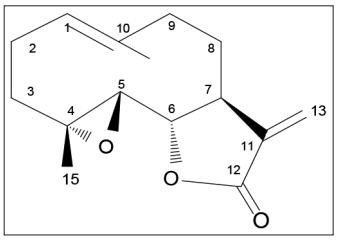

Figure 3: Structural formula of parthenolide

Comparative data on the immunomodulatory activity of trilobolide (Figure 4) and thapsigargin (Figure 5) showed that samples of terpenoids and their derivatives with acyl groups in positions C-3, C-8 and C-10, as well as in the presence of at least one free hydroxyl group, retain activity [6]. Stimulating activity is accompanied by the secretion of cytokines IL-1β and IL-6, vascular endothelial growth factor and granulocyte-macrophage colony-stimulating factor. It was found that trilobolide (Figure 4), thapsigargin (Figure 5), and 2-acetoxytrilobolide (Figure 6) stimulate the production of cytokines, whereas 2-hydroxy-10-deacetyltrilobolide (Figure 7) did not affect the production of cytokines. Thus, in addition to the formation of the 7,11-diol, ester substituents at the C-2 and C-10 positions (or absent from the C-2 of trilobolide) (Figure 4) are important

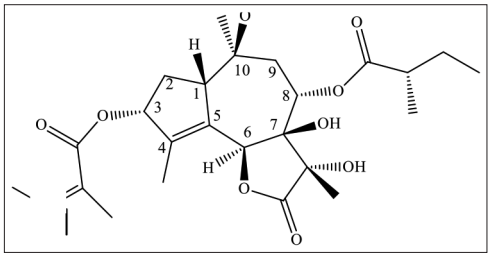

Figure 4: Structural formula of trilobolide

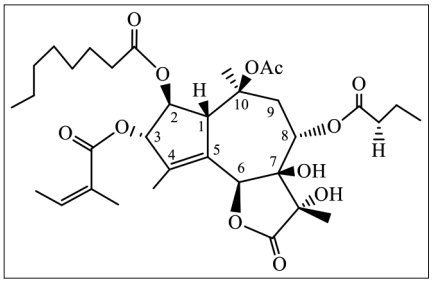

Figure 5: Structural formula of thapsigargin

for the immunostimulatory activity, i.e. secretion of cytokines and NOS. If they are replaced by hydroxyl groups (as in compound 7), then the ability to secrete cytokines completely disappears [7], [8].

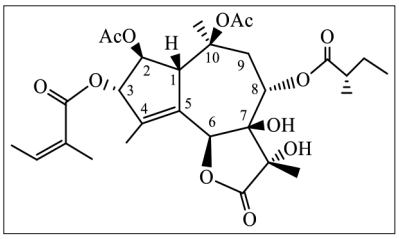

Figure 6: Structural formula of 2-acetoxytrilobolide

The relationship between the molecular structure and immunobiological activity of compounds (4–5) has been studied. Acetylation at the 7-OH and 11-OH positions of the lactone ring or acyl modification of the guaianolide functional groups (including relactonization) of trilobolide (Figure 4) results in an inability to stimulate cytokine secretion and NOS. It was found that minor structural changes achieved by catalytic hydrogenation or hydrogenolysis retained the original trilobolide immunoactivity (Figure 4). It has been confirmed that the influence of the lactone vicinal diol (glycol) group in combination with other structural functions is necessary for the immune properties of the trilobolide or thapsigargin type of guaianolides [8].

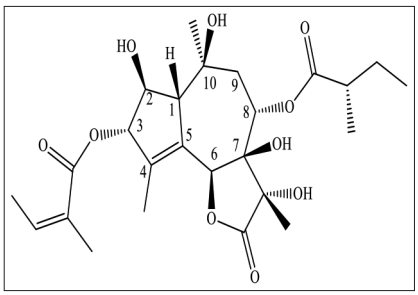

Figure 7: Structural formula of 2-hydroxy-10-deacetyltrilobolide

At the same time, it should be borne in mind that acute and chronic inflammation differ significantly not only in the duration of the reaction but also in the peculiarity of their cellular and molecular elements. Acute inflammation ends within a few days, and immune disorders are usually transient. The development of persistent immunodeficiency states, which are of the greatest practical interest, is characteristic of chronic, often recurrent infectious and non-infectious diseases.

Thus, epoxyguaianolide (Figure 1) acts on two phases of inflammation: Exudation and proliferation. The use of this substance, apparently, leads to a decrease in the permeability of the vascular wall, which contributes to the suppression of the exudative phase of inflammation, a decrease in the activity of other inflammatory mediators, the division of fibroblasts, and collagen synthesis, which prevents the development of proliferative processes. The pronounced anti-inflammatory effect of epoxy-guaianolide (Figure 1) is confirmed by the literature data, which indicate that many sesquiterpene lactones suppress the exudative and proliferative components of the inflammatory response [19], [20], [21].

Based on the results of the experiments, the inhibitory effect of sesquiterpene lactones on the transcription factor NF-KB, which is a critical point of intersection of a number of signaling pathways, including those, leading to the synthesis of pro-inflammatory cytokines, was established. NF-KB activation requires its dissociation from inhibitory proteins. It is this dissociation that is activated by IL-1 and tumor necrosis factor-α. After dissociation, free NF-KB is transported to the nucleus, where it activates genes for proteins involved in immune and inflammatory responses. Therefore, the anti-inflammatory and immunomodulating effect of many sesquiterpene lactones is carried out through the effect on transcription factors.

Analyzing the possible mechanism of action of epoxyguaianolide (Figure 1), as well as the presence of common damaging factors and mediators of immune and inflammatory response cells, indicate the universal nature of pathogenetic mechanisms in various immune-inflammatory processes. It is known that stimulation of the cellular-humoral link causes an increase in anti-inflammatory effects; therefore, in some cases, the use of immunostimulants is advisable to use in therapy to induce anti-inflammatory effects.

The immunomodulatory properties of epoxy-guaianolide (Figure 1) were studied in intact animals and a model of cyclophosphamide immunosuppression in comparison with the hydrochloride of its dimethylaminoderivative (Figure 2) and the well-known immunomodulator levamisole.

The introduction of epoxy-guaianolide (Figure 1) in all doses did not lead to a significant change in immunological parameters in all observed periods. When studying the effect of epoxy-guaianolide (Figure 1) on the leukogram of the peripheral blood of animals, no significant changes in the number of leukocytes and lymphocytes were found in comparison with control animals.

The use of epoxy-guaianolide (Figure 1) at doses of 5–10 mg/kg did not lead to a significant change in the number of B-lymphocytes, although there was a slight downward trend. In contrast to epoxy-guaianolide (Figure 1), in the group of animals where

the hydrochloride of dimethylaminoderivative (Figure 2) was used, the level of B-cells steadily decreased during the experiment.

Although both trilobolide (Figure 4) and thapsigargin (Figure 5) do not show any selectivity for normal and inflammatory cells, they are therefore considered to be monoclonal antibody conjugates.

In contrast to dimethylaminoepoxyguaianolide hydrochloride (Figure 2), where a pronounced increase in null cells was noted, the administration of epoxyguaianolide (Figure 1) at doses of 5 and 10 mg/kg had an insignificant increase in the number of null lymphocytes.

The introduction of epoxyguaianolide (Figure 1) in all doses did not lead to a significant change in the absolute percentage of T-lymphocytes in all observed periods. The level of theophylline-resistant and theophylline-sensitive subpopulations of T-lymphocytes remained unchanged throughout the experiment.

When using dimethylamino derivative hydrochloride (Figure 2), the number of T-lymphocytes was high throughout the entire course of administration and remained above the control value, and at the end of the administration, a significant decrease in the relative number of T-suppressors was observed by day 15; the content of T-helpers increased by day 10 and remained at a high level after the end of the course; the level of B-cells during the experiment steadily decreased; there was a pronounced increase in null cells.

The intensity of the hemagglutination reaction and the delayed-type hypersensitivity reaction slightly increased in all animals treated with epoxy-guaianolide (Figure 1) and its derivative (Figure 2), and it was most pronounced with the introduction of the derivative.

In the observed periods (5, 10, and 15 days), the indicators of the spontaneous nonstress test (NST) using epoxy-guaianolide (Figure 1) at a dose of 5–10 mg/kg and its derivative (Figure 2) did not differ from those of the control group. When analyzing the ability of the studied cells to activate, a significant excess of the indices of the induced NST test over the spontaneous one was determined.

In the study of the effect of epoxy-guaianolide (Figure 1) and its dimethylaminoderivative hydrochloride (Figure 2) on the activity of phagocytosis, no significant effect was noted.

Analysis of the obtained experimental data on the effect of epoxy-guaianolide (Figure 1) on the ratio of different populations of lymphocytes did not reveal a definite nature of the response to the administration of epoxy-guaianolide (Figure 1) in intact animals. The use of dimethylaminoepoxyguaianolide hydrochloride (Figure 2) contributed to a significant decrease in the content of B-cells, as well as theophylline-sensitive T-lymphocytes. The level of the theophylline-resistant subpopulation of T-lymphocytes increased by day 10 and remained at a high level after the end of the course.

On the model of cyclophosphamide immunosuppression, it was found that epoxy-guaianolide (Figure 1) has a pronounced immunomodulatory effect.

The use of epoxy-guaianolide (Figure 1) contributed to a decrease in the level of leukopenia and lymphopenia, compared with animals that did not receive the drug. From the 5th day of administration, a significant increase in the number of leukocytes and lymphocytes was noted. By day 10, the indicators of epoxy-guaianolide (Figure 1) at a dose of 10 mg/kg approached the values of intact animals and at a dose of 5 mg/kg - by day 15. When using levamisole, complete correction of the reduced number of leukocytes was not observed. At the same time, the therapeutic effect of epoxy-guaianolide (Figure 1) at doses of 5 and 10 mg/kg was higher than that of the reference drug and dimethylaminoderivative hydrochloride (Figure 2). The use of epoxy-guaianolide (Figure 1) at a dose of 2.5 mg/kg did not have a particular effect on the level of leukopenia and lymphopenia (Table 1).

When observing the number of B-lymphocytes in mice with cyclophosphamide immuno-depression treated with epoxyguaianolide (Figure 1) at all doses and the hydrochloride derivative, a clear trend towards an increase in the reduced number of B-cells was revealed. So, on day 5, epoxyguaianolide (Figure 1) at a dose of 5–10 mg/kg and at a dose of 10 mg/kg by days 10 and 15 significantly exceeded the hydrochloride derivative and the reference drug. The use of epoxy-guaianolide (Figure 1) at a dose of 2.5 mg/kg did not have a special immunomodulatory effect in all observed periods.

In general, the analysis of the number of B-lymphocytes in dynamics showed a positive effect of the use of (Figure 1) at a dose of 5–10 mg/kg, but the initial level of B-cells was not restored. Oral administration of epoxy-guaianolide (Figure 1) at doses of 5–10 mg/kg caused a significant decrease in O-cells in comparison with the hydrochloride derivative already on the 5th day. On the 10th day, there was a decrease in the percentage of O-lymphocytes, with a gradual alignment to the indicators of intact animals, apparently due to an increase in B-lymphocytes. In the group of animals where epoxy-guaianolide (Figure 1) was used at a dose of 2.5 mg/kg, there was practically no decrease in O-cells (Table 1).

Epoxyguaianolide (Figure 1) and dimethylaminoepoxyguaianolide hydrochloride (Figure 2) had a pronounced effect on the state of the T-cell system. In dynamics, a clear dose-dependent effect can be traced. On day 10, epoxy-guaianolide (Figure 1) at a dose of 10 mg/kg contributed to the complete correction of the relative number of T-lymphocytes and the T-helper subpopulation. At the same time, the number of T-suppressors did not reach the indices of intact animals, and in the observed

Table 1: The effect of epoxy-guaianolide on immunological parameters in the model of cyclophosphamide immunosuppression

Day	Epoxyguaianolide (Figure 1)			Dimethylaminoepoxyguaianolide hydrochloride (Figure 2)	Levamisole	Control
	2.5	5	10			
01	2	3	4	5	6	7
The content of leukocytes, x10^9/mL						
Before	5.3 ± 0.3 (intact-12.0 ± 0.3)			6.9 ± 0.4		
5	6.3 ± 0.2	8.0 ± 0.4	8.4 ± 0.6	8.3 ± 0.4	6.0 ± 0.2	5.6 ± 0.3
10	7.8 ± 0.4	9.7 ± 0.6	11.2 ± 0.4	10.5 ± 0.4	8.6 ± 0.7	6.9 ± 0.4
15	10.0 ± 0.2	11.0 ± 0,2	11.7 ± 0.3	6.9 ± 0.4	9.8 ± 0.3	9.0 ± 0.5
5	6.3 ± 0.2	8.0 ± 0.4	8.4 ± 0.6		6.0 ± 0.2	5.6 ± 0.3
Content of lymphocytes, ×10^9/mL (%)						
Before	3.0 ± 0.3 (64%) intact-12.0 ± 0.3 (71%)					
1	2 (%)	3 (%)	4 (%)	5 (%)	6 (%)	7 (%)
5	4.21 ± 0.13	5.42 ± 0.11*	5.80 ± 0.24*	4.63 ± 0.43*	4.03 ± 0.56	3.64 ± 0.23
	66	68	70	67	67	65
10	5.30 ± 0.63	6.89 ± 0.58*	8.06 ± 0.42*	5.81 ± 0.23*	5.93 ± 0.22	4.62 ± 0.35
	68	71	72	70	70	66
15	6.65 ± 0.31	8.03 ± 0.40*	8.77 ± 0.20*	7.45 ± 0.31*	6.95 ± 0.36	6.21 ± 0.34
	69	73	75	71	71	69
The content of T-lymphocytes. x10^9/mL (%)						
Before	1.02 ± 0.07 (23%) intact – 3.01 ± 0.09 (35%)					
5	1.02 ± 0.09	1.43 ± 0.10	1.57 ± 0.19	1.19 ± 0.07	1.56 ± 0,04	0.86 ± 0.10
	24	26	31	26	27	24
10	1.38 ± 0.09	2.01 ± 0.18	2.63 ± 0.12	1.62 ± 0.07	2.14 ± 0.12	1.13 ± 0.11
	26	29	33	28	31	25
15	2.01 ± 0.13	2.49 ± 0.19	3.07 ± 0.19	2.23 ± 0.16	3.03 ± 0.10	1.61 ± 0.15
	28	31	35	30	35	26
Subpopulations of T-lymphocytes (%)						
Before	Tx 0,11 ± 0.07 (16%) intact – 0.81 ± 0.12 (29%), Tc 0.06 ± 0.002 (6%) intact 0.37 ± 0.11 (12%)					
	Tx	Tc	Tx	Tc	Tx	Tc Tx Tc Tx Tc Tx Tc
5	18	6	20	6	20	7 19 6 19 6 17 6
10	19	6	21	7	23	7 20 7 21 6 18 6
15	21	7	24	8	25	8 23 8 24 8 19 7
The content of B-lymphocytes, x10^9/ml (%)						
Before	0.28 ± 0.04 (10%) intact - 1.90 ± 0.06 (24%)					
5	0.48 ± 0.09	0.79 ± 0.08*	0.92 ± 0.09*	0.53 ± 0.06	0.50 ± 0.07	0.35 ± 0.08
	11	15	16	12	12	10
10	0.79 ± 0.06	1.18 ± 0.05*	1.55 ± 0.20*	0.90 ± 0.10	0.91 ± 0.14	0.55 ± 0.08
	15	17	19	16	15	12
15	1.19 ± 0.12	1.57 ± 0.10*	1.84 ± 0.09*	1.23 ± 0.07	1.25 ± 0.13	0.80 ± 0.04
	17	20	21	17	18	13
The content of O-lymphocytes, x10^9/mL (%)						
Before	1.80 ± 0.12 (59%) intact – 2.70 ± 0.10 (32%)					
5	2.24 ± 0.15	2.66 ± 0.12*	2.73 ± 0.13*	2.43 ± 0.07	2.06 ± 0.10	2.78 ± 0.35
	53	49	47	52	51	56
10	2.55 ± 0.12	2.90 ± 0.11*	3.30 ± 0.32*	2.71 ± 0.16	2.59 ± 0.18	2.41 ± 0.16
	48	42	42	47	44	52
15	3.10 ± 0.17	3.13 ± 0.43	3.30 ± 0.32*	3.20 ± 0.37	2.78 ± 0.35	3.09 ± 0.42
	45	39	35	34	40	50

Ote: The results of 8 observations in each group *-the significance of differences with the comparison drug $p < 0.05$.

periods of complete recovery of this subpopulation did not occur. In all observed periods, epoxy-guaianolide (Figure 1) at a dose of 5 mg/kg was somewhat superior in effect to the hydrochloride derivative. In the group of animals where epoxyguaianolide was used at a dose of 2.5 mg/kg, it did not have a pronounced effect on the state of the T-population. When using epoxy-guaianolide (Figure 1) at a dose of 5 mg/kg, a comparable effect with levamisole was noted. The number of T-lymphocytes and T-helpers in animals, where epoxy-guaianolide was used at a dose of 5 mg/kg, slightly exceeded the effect of the hydrochloride derivative (Figure 2). The effect of epoxy-guaianolide (Figure 1) at a dose of 10 mg/kg was significantly higher than that of dimethylaminoepoxyguaianolide hydrochloride (Figure 2). According to the results of the experiments, it was found that the use of epoxy-guaianolide (Figure 1) at a dose of 10 mg/kg had a corrective effect, contributing to a complete correction of the number of T-lymphocytes, an increase in the level of T-helpers and, to a lesser extent, T-suppressors. When using epoxy-guaianolide (Figure 1) and its derivative (Figure 2), a distinct increase in the reduced number of B-cells was revealed, but there was no recovery of the initial level in the observed periods. It should be noted that the effect of epoxy-guaianolide (Figure 1) was significantly higher than that of dimethylaminoderivative hydrochloride (Figure 2) (Table 1).

Epoxyguaianolide (Figure 1) and its water-soluble hydrochloride derivative (Figure 2) had virtually no significant effect on phagocytosis compared to the reference drug levamisole. At the same time, the digital values slightly exceeded the control indicators.

In the group of untreated animals, no complete recovery of the quantitative and functional characteristics of the cells of the immune system was registered by day 15.

An analysis of the obtained results, shown in Table 1, indicates that in animals with a model of cyclophosphamide immunosuppression, severe disorders of the immune status were noted in the form of severe leukopenia and lymphopenia. Severe disorders were observed in the T-population, where, against the background of a decrease in the absolute and relative number of T-lymphocytes, the level of T-helpers and T-suppressors decreased. A decrease in the number of B-lymphocytes and an increase in the level of O-lymphocytes were established.

The comparison drug levamisole had a pronounced therapeutic effect, which by the 15th day

was manifested in an increase in the number of leukocytes, correction of lymphopenia. The best effect was noted in relation to the correction of the number of T-lymphocytes and their subpopulations of T-helpers, the normalization of the NST test. At the same time, there was a slight effect on the correction of the number of B-lymphocytes (Table 1).

Epoxyguaianolide (Figure 1) and dimethylaminoepoxyguaianolide hydrochloride (Figure 2) have a dose-dependent effect on cellular and humoral immune responses. At the same time, it is noted that epoxy-guaianolide (Figure 1) at a dose of 2.5 mg/kg, in general, had a weak therapeutic effect.

The use of epoxy-guaianolide (Figure 1) contributed to a decrease in the level of leukopenia and lymphopenia. From the 5th day of the introduction of the sample, a significant increase in the number of leukocytes and lymphocytes was noted. By day 10, the indicators of epoxy-guaianolide (Figure 1) at a dose of 10 mg/kg approached the values of intact animals, and at a dose of 5 mg/kg, by day 15. When using levamisole, complete correction of the reduced number of leukocytes was not observed. At the same time, in all observed periods, the therapeutic effect of epoxy-guaianolide at doses of 5 and 10 mg/kg was higher than that of the reference drug and dimethylaminoepoxyguaianolide hydrochloride (Figure 2).

Epoxyguaianolide (Figure 1) and dimethylaminoderivative hydrochloride (Figure 2) had a pronounced effect on the state of the T-cell system. On day 10, epoxy-guaianolide (Figure 1) at a dose of 10 mg/kg contributed to the complete correction of the relative number of T-lymphocytes and the T-helper subpopulation. At the same time, the number of T-suppressors did not reach the indices of intact animals, and the complete recovery of the subpopulation did not occur in the observed periods. In all observed periods, epoxy-guaianolide (Figure 1) at a dose of 5 mg/kg was superior in effect to dimethylaminoepoxyguaianolide hydrochloride (Figure 2). In the group of animals where it was used, epoxy-guaianolide (Figure 1) at a dose of 2.5 mg/kg did not have a pronounced effect on the state of the T-population. When using epoxyguaianolide (Figure 1) at a dose of 5 mg/kg, a comparable effect was observed with the reference drug levomisole. The number of T-lymphocytes and T-helpers in animals, where epoxy-guaianolide (Figure 1) was used at a dose of 5 mg/kg, slightly exceeded the effect of dimethylaminoepoxyguaianolide hydrochloride (Figure 2). The effect of epoxyguaianolide (Figure 1) at a dose of 10 mg/kg was significantly higher than that of the compound (Figure 2). The use of epoxy-guaianolide (Figure 1) at a dose of 10 mg/kg had a corrective effect, contributing to the complete correction of the number of T-lymphocytes, an increase in the level of T-helpers and, to a lesser extent, T-suppressors. When using compounds (Figures 1 and 2), a clear increase in the reduced number of B-cells was revealed, but recovery of the initial level in the observed periods was not revealed. It should be noted that the effect of epoxy-guaianolide (Figure 1) is significantly higher than that of dimethylaminoepoxyguaianolide hydrochloride (Figure 2).

The selectivity of the action of natural epoxy-guaianolide due to the exomethylene group conjugated with the carbonyl of γ-lactone, actively interacting with the sulfhydryl or amino group of enzymes, such as farnesyl protein transferase, that is, it selectively inhibits the mitosis of tumor cells without affecting healthy tissue. When dimethylaminoepoxyguaianolide hydrochloride (Figure 2) is injected into the body, hydrolytic deamination occurs (Figure 2) with the formation of the original natural epoxy-guaianolide (Figure 1).

The administration of epoxy-guaianolide (Figure 1) at doses of 5–10 mg/kg caused a significant decrease in the number of O-cells by day 5, with a gradual approach to healthy animals by day 10, which is associated with an increase in the level of B-lymphocytes. In the group of animals where epoxy-guaianolide (Figure 1) was used at a dose of 2.5 mg/kg, a decrease in O-cells was not observed.

On the 5th day of treatment with epoxyguaianolide (Figure 1) and dimethylaminoguaianolid hydrochloride (Figure 2) in all doses, hemagglutinin titers and the intensity of the delayed-type hypersensitivity reaction (DHRT) of the group of untreated animals exceeded. The most pronounced reaction of the immune system was observed on the 10th day of treatment. Thus, under the action of epoxy-guaianolide at a dose of 5 mg/kg, hemagglutinin titers significantly exceeded those in the group of animals that used (Figure 2). The intensity of DHRT increased in all animals, and it was most pronounced with the introduction of epoxyguaianolide at a dose of 10 mg/kg. In all the observed periods, the indicators of the spontaneous NST test in the group of animals with epogguaianolide at a dose of 5 mg/kg had a stimulating effect comparable to the action of dimethylaminoguaianolide hydrochloride (Figure 2). Whereas epoxyguaianolide (Figure 1) at a dose of 10 mg/kg significantly exceeded the effect of compound (Figure 2). When analyzing the ability of the studied cells to activate, a significant excess of the indices of induced NST over the spontaneous NST test was determined. Thus, by the 10th day, the indicators of epoxy-guaianode (Figure 1) at a dose of 10 mg/kg approached the values of intact animals and were comparable with levamisole in terms of the severity of the therapeutic effect. Analysis of the results obtained indicates the presence of pronounced immunostimulatory properties in epoxy-guaianolide (Figure 1).

Conclusions

The presented experimental data demonstrate a rather high anti-inflammatory activity of 1(10) β-epoxy-5,7α,6β(H)-guai-3(4),11(13)-dien-6,12-olide (hereinafter referred to as epoxy-guaianolide) not only in acute, but also in chronic inflammation. Given that chronic inflammation in diseases has a long life cycle and the leading role is played by actively phagocytic macrophages, polymorphonuclear leukocytes, monocytes, T-lymphocytes and other cells that form a granuloma, our results obtained from experiments with epoxy-guaianolide (Figure 1) indicate that it has pronounced immunomodulatory properties and, in combination with anti-inflammatory properties, has a high therapeutic effect.

The above experimental results allow us to classify the studied epoxyguaianolide (Figure 1) as an immunomodulator, since, when interacting with the cells of the immune system, epoxy-guaianolide (Figure 1) activated the studied parameters in animals with immunosuppression, and in intact animals it did not affect the above parameters. Such properties of epoxy-guaianolide (Figure 1) exclude the minimal possibility of hyperactivation of the immune system, which is an important condition for its use as an immunotropic agent.

Sufficiently high immunomodulatory and anti-inflammatory properties of epoxyguaianolide (Figure 1), as well as modern concepts of the pathogenesis of inflammatory and immune processes, indicate its possible complex effect on the inflammatory process.

Based on the foregoing, epoxy-guaianolide (Figure 1) is recommended for clinical trials as an immunomodulatory agent in the treatment of chronic inflammatory diseases accompanied by secondary immunodeficiencies.

Institutional Review Board Statement

Animal study protocol approved by the Bioethics Committee of the NCJSC “Karaganda Medical University” (No. 4 dated 06 December 2021).

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