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Translation

European Patent No. 0 346 501

Description

The present invention relates to a field of pharmacy and, in particular, concerns a new medicament for the treatment of immunodeficient conditions.

State of the Art on which the invention is based

Medicaments for the treatment of immunodeficient conditions, in particular thymus preparations, are known which may be obtained from raw animal material, e.g. thymosin of Fraction 5 (Goldstein A. L., Guha A., Latz M. M., Hardy H. A., White A., Proc, Nat. Acad. Sci, USA, 1972, v. 69, p. 1800-1803), thymalin (CH, A, 659586), tactivin (US, A, 4377511). These preparations consist of a complex of materials of polypeptide nature, which are capable of regulating different stages of the proliferation and differentiation of T-lymphocytes. However, the practical use of such preparations is significantly hindered because the method for their production is complicated, the yield of active substances is low and the physico-chemical and biological properties of these active substances are variable. In addition, patients sometimes develop secondary reactions when they are used, because ballast components are present in the natural thymus preparations.

One of the most effective remedies for treating immunodeficient conditions is thymalin, which is a complex of polypeptides with a molecular mass of 600 to

6000 D in a pharmaceutical solvent, for example glycine*. It is introduced once a day within 5 days in a dose of 10 mg of active substance. The content of active components in the given preparation means that it needs to be used in high doses (up to 50 mg per course of treatment).

The object of the present invention is to develop such a medicament for the treatment of immunodeficient conditions, which is highly effective whilst exhibiting low toxicity and does not produce any secondary reactions.

The invention sets out to develop a medicament for the treatment of immunodeficient conditions, which contains such an active substance of peptide nature, which brings about an increase in the biological effectiveness of the preparation, causes low toxicity and does not produce any secondary reactions.

This object is achieved in that, in accordance with the invention, the medicament for the treatment of immunodeficient conditions, which provides an active substance of peptide nature and a pharmaceutical diluent, contains a peptide of the following structure as the active substance of peptide nature:

H-L-Glu-L-Trp-OH.

The medicament of the invention for the treatment of immunodeficient conditions is highly effective in a dose which is 50 to 500 times lower than that of thymalin and has a distinct immuno-stimulating effect in the case of

*Footnote: The German word "Glyzin", which I have translated as "glycine", appears to be a mistake and the agents have confirmed that this should read "Glyzerin" (glycerin).

radiation immunodeficiency. Under the action of the medicament, a significant stimulation of the production of lymphocytes and T-lymphocytes and a distinct modulation of the relative proportion of the subpopulations of the immuno-competent cells is detected.

It was not possible to establish the LD_{50} of the medicament of the invention because the minimum lethal dose was not detected in any animal when the therapeutic dose was increased 1000 times (1 mg/kg).

The preparation of the invention does not give rise to any secondary effects.

In accordance with the invention, it is advisable that, when in the form of an injection solution, the medicament contains the active substance in a quantity of 0.001 to 0.01 % by weight.

In accordance with the invention, it is advisable that the medicament contains a 0.9% aqueous sodium chloride solution and/or a 0.5% Novocaine solution as the pharmaceutical diluent.

In accordance with the invention, it is advisable that, when in the form of tablets, suppositories or capsules, the medicament contains the active substance in a quantity of 0.1 mg per one tablet, suppository or capsule.

In accordance with the invention, it is advisable that, when in the form of tablets, suppositories or capsules, the medicament contains a filling agent - starch, glucose, glycine* - as the pharmaceutical diluent.

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*Footnote: see footnote on page 2.

Other aims and advantages of the invention are explained by the following detailed description of the medicament for the treatment of immunodeficient conditions.

The medicament of the invention for the treatment of immunodeficient conditions contains the peptide of the following structure H-L-Glu-L-Trp-OH and a pharmaceutical diluent.

The said peptide can be obtained in the solution according to the known two-stage chemical synthesis, starting out from the active ester of the protected L-glutamic acid derivative and L-tryptophan derivative. After removal of the protective groups and chromatographic purification, the peptide is lyophilised and is obtained in the form of an amorphous powder.

The peptide of the structure H-L-Glu-Trp-OH is a white lyophilised powder which is easily soluble in water and dimethylformamide and insoluble in chloroform and ether. $[\alpha]_D^{22}$ = 12.6; C = 0.5, H_2O. R_f = 0.65 (butanol: acetic acid: water = 3:1:1). The maximum adsorption in the UV range is at 275±5 nm. In the H'-NMR-spectrum, the following signals for the corresponding amino acid residues at 500 MHz for a 0.001 mol/l peptide solution are present.
Trp - 3.17; 3.37; 4.57; 7.16; 7.24; 7.71; 7.49;
Glu - 1.90; 1.96; 2.21; 3.72.

The stated data clearly confirms the structure of the synthetised compound - the active substance of the medicament of the invention.

The active substance of the medicament of the invention can be used both in the form of the free peptide and in the form of water-soluble salts, for example sodium, potassium, ammonium and zinc salts.

The preparation of the invention can be used in different galenical forms, for example in tablets, solutions (intranasal and injection solutions), drops, ointments and suppositories. The preparation of the invention is preferably used in the form of injection solutions with a content of active substance of 0.001 to 0.01 % by weight (0.0001 to 0.001 mg/kg body mass or 10 to 100 µg active substance in 1 ml solvent). As the pharmaceutical diluent for the injection method, the preparation contains any pharmaceutically suitable solvent, for example 0.9% aqueous sodium chloride solution, distilled water, Novocaine solution for injections, Ringer's solution, glucose solution.

When in the form of tablets and suppositories, the preparation of the invention preferably contains the active substance in a quantity of 0.1 mg per one tablet or suppository. It is advisable to use starch, glucose or glycine* as the pharmaceutical filling agent for tablets. Any pharmaceutically suitable base can be used as the pharmaceutical filling agent for suppositories.

The medicament of the invention is highly effective in the treatment of immunodeficient conditions, has a strong effect on the immunological reactivity of the organism and contributes to a significant stimulation of the production of lymphocytes and T-lymphocytes and to a distinct modulation of the relative proportion of the subpopulations of the immuno-competent cells.

The preparation does not display any toxicity, is introduced in small individual or treatment doses and has a wide range of therapeutic action. The preparation of the invention can be used widely in medicinal

*Footnote: see footnote on page 2.

practice for the treatment and prophylaxis of a number of human diseases.

The preparation of the invention for the treatment of immunodeficient conditions was tested on animals and, in hospitals, on humans.

The biological effectiveness of the dipeptide-based preparation of the invention was tested in comparison with the effectiveness of thymalin in the case of secondary immunodeficient conditions, in particular induced by radiation. The tests were carried out on male guinea pigs of 150 to 200g body mass. In the installation, the animals were exposed to radiation in a dose of 1 Gr. One day after radiation, thymalin and the preparation of the invention were introduced intramuscularly to the test groups (up to 10 guinea pigs in each group) in a dose of 0.0001 to 0.001 mg/kg in 0.5 ml of 0.9% aqueous sodium chloride solution over a period of 3 days. The control animals were given 0.5 ml of 0.9% aqueous sodium chloride solution according to a similar pattern. 10 days after radiation, the number of karyocytes, T-lymphocytes and B-lymphocytes in the thymus and the spleen was determined by the rosette formation method.

The results obtained are given in tables 1 and 2.

It was established that, in a dose of 0.001 mg/kg, dipeptide has an immuno-stimulating effect in that it increases the number of karyocytes and T-lymphocytes in the thymus of the animals exposed to radiation by more than three times. At the same time, in a dose of 0.001 mg/kg, dipeptide normalizes the content of B-lymphocytes in the thymus and spleen of the animals exposed to radiation. In all the tested doses, thymalin does not have any reliable influence on the cell situation in the

thymus and spleen of the animals exposed to radiation.

On the basis of the results obtained under similar conditions in the additional series of tests, it was established that thymalin only has an immuno-stimulating effect in a dose of 0.1 mg per kg body mass of the animal (Table 3).

Table 1.

Comparative test of the effect of the preparation of the invention and thymalin on the cells in the thymus of the guinea pigs exposed to radiation (X±m)

Animal Group	number of karyocytes (T/mg)	number of T-lymphocytes (T/mg)	number of B-lymphocytes (T/kg)
Intact group	481.4±21.3	378.5±25.5	4.8±0.3
Control group (radiation)	87.4±13.2	49.6±5.6	5.7±0.6
Dipeptide (0.00001 mg/kg)	89.3±10.7	51.3±6.7	5.3±0.5
Dipeptide (0.0001 mg/kg)	139.8±14.1^{x}	89.4±9.1^{x}	4.9±0.5^{x}
Dipeptide (0.001 mg/kg)	275.8±26.4^{x}	194.7±21.3^{x}	3.3±0.4^{x}
Thymalin (0.00001 mg/kg)	85.4± 11.6	53.6±7.2	5.4±0.6
Thymalin (0.0001 mg/kg)	90.4±9.7	46.3±5.7	5.3±0.5
Thymalin (0.001 mg/kg)	89.1±13.6	47.9±6.1	5.7±0.7

x - statistically significant ($P < 0.05$) in comparison with the control group

Table 2.

Comparative test of the effect of the preparation of the invention and thymalin on the cells in the spleen of the guinea pigs exposed to radiation (X±m)

Animal group	Number of karyocytes (T/mg)	Number of T-lymphocytes (T/mg)	Number of B-lymphocytes (T/mg)
Intact group	409.3±25.6	40.4±2.9	72.5±5.4
Control group (Radiation)	147.9±23.2	29.8±3.4	51.4±4.3
Preparation of the invention (0.00001mg/kg)	150±15.8	31.2±3.6	50.9±5.4
Preparation of the invention (0.0001mg/kg)	148.3±16.0	30.4±3.9	62.4±6.1[x]
Preparation of the invention (0.001mg/kg)	146.6±18.1	29.9±3.3	73.2±5.1[x]
Thymalin (0.00001mg/kg)	151.4±16.7	32.0±4.1	53.7±6.3
Thymalin (0.0001mg/kg)	147.3±15.1	31.7±5.2	49.7±5.8
Thymalin (0.001mg/kg)	150.0±16.6	28.9±4.1	54.7±5.2

x - statistically significant ($P < 0.05$) in comparison with the control group

Table 3

Influence of thymalin on the cells in the thymus and the spleen of the guinea pigs exposed to radiation (X±m)

Animal group	Number of karyocytes (T/mg)	
	Thymus	Spleen
1	2	3
Intact group	481.4±21.3	409.3±25.6
Control group (radiation)	87.4±13.2	147.9±23.2
Thymalin (0.01 mg/kg)	89.3±11.6	153.6±17.1
Thymalin (0.05 mg/kg)	101.4±15.3	149.4±16.0
Thymalin (0.1 mg/kg)	280.7±27.3^{x}	154.3±16.1

Continuation of Table 3

Animal Group	Number of karyocytes (T/mg)			
	Thymus	Spleen	Thymus	Spleen
1	2	3	4	5
Intact group	378.5±25.5	40.4±2.9	4.8±0.3	72.5±5.4
Control group (Radiation)	49.6±5.6	29.8±3.4	5.7±0.6	51.4±4.3
Thymalin (0.01 mg/kg)	51.3±7.8	33.6±5.7	5.9±0.7	54.5±6.3
Thymalin (0.05 mg/kg)	73.7±14.1	26.4±4.1	5.1±0.6	61.7±9.0
Thymalin (0.1 mg/kg)	206.7±19.8^{x}	30.1±3.3	3.9±0.5^{x}	75.8±6.2^{x}

x - statistically significant ($P < 0.05$) in comparison with the control group

Consequently, the preparation of the invention has a marked immuno-stimulating effect in the case of radiation immunodeficiency, in a dose 50 to 500 times lower than thymalin.

A comparative assessment was conducted with regard to the influence of the preparation of the invention and thymalin on the expression of the receptors of the thymocytes developed by intact guinea pigs. It is known that, after the lymphocytes have been treated with trypsin, the majority of E-receptors on the cell surface are destroyed; there is consequently a decrease in the percentage of T-lymphocytes (also known as E-RFCs in English) determined in the rosette formation reaction. The addition of the thymus preparations to the lymphocytes treated with trypsin leads to the restoration of the destroyed E-receptors, which is reflected in an increase in the percentage of E-RFCs (T-lymphocytes).

The preparation of the invention and thymalin were added to the thymocytes developed by 10 intact guinea pigs in doses of 0.0001 to 1 μg/ml. The results of the test are shown in Table 4.

Table 4

Influence of the preparation of the invention and thymalin on the expression of the R-receptors of thymocytes (X±m).

Preparation	Ea - RFC (%)	P
1	2	3
Control	36.1±3.1	-
Preparation of the invention (0.0001μg/ml)	37.4±4.0	> 0.05
Preparation of the invention (0.001μg/ml)	47.4±5.1	> 0.05
Preparation of the invention (0.01μg/ml)	61.4±5.3	< 0.05
Preparation of the invention (1μg/ml)	62.7±6.1	< 0.05
Thymalin (0.0001μg/ml)	35.3±3.9	> 0.05
Thymalin (0.001μg/ml)	39.6±4.1	> 0.05
Thymalin (0.01μg/ml)	38.7±4.0	> 0.05
Thymalin (1μg/ml)	57.0±4.7	< 0.05

It was established that both the preparation of the invention and thymalin restored E-receptors in the thymocytes treated in the trypsin. The preparation of the invention is effective in doses of 0.01 to 1μg/ml. Thymalin is effective in doses of at least 1μg/ml.

The clinical-immunological effectiveness of the medicament of the invention was assessed in 300 patients with purulent inflammatory diseases of the bones and soft tissue (chronic post-traumatic osteomyelitis of the long tubular bones, purulent inflammatory complications of trauma and operations in diseases of the supporting locomotor system, acute and chronic purulent inflammatory diseases of the bones and soft tissue of the jaw and facial region, staphylococcal pyodermas).

The medicament of the invention was introduced intramuscularly or intranasally in single doses of 100 μg over 3-10 days (on average 500μg of preparation per course of treatment). 200 patients of similar pathology formed the control group.

In the patients with the purulent inflammatory diseases of the bones and soft tissue in various locations, there was a reduction by various degrees of quantitative and in particular functional indexes of cell immunity and unspecific resistance. The disruption of the differentiation of the subpopulations of T-cells, i.e. a reduction in the number of T-helper cells ($OKT4^+$) and T-suppressors (OKT8+), was also detected.

The use of the medicament of the invention in complex treatment contributed to improving the clinical progress of the disease or to curing the disease in 75.3 to 91.7% of the patients, which allowed faster cleaning and healing of the wound, reduced the surface of destruction of the bone tissue, meant that purulent inflammatory skin diseases could be arrested more quickly and reduced the duration of treatment by 20 to 30% in comparison with the control patients. The clinical effectiveness of the medicament of the invention was accompanied by the restoration of the quantitative and functional indices of the T-system of immunity and unspecific resistance.

Toxic and allergic reactions were not recorded in any case of clinical use of the preparation of the invention.

A comparative assessment of the influence of the preparation of the invention and thymalin on the subpopulations of lymphocytes was carried out for patients of different pathologies: on the T-helper cells ($OKT4^+$), T-suppressors ($OKT\ 8^+$), the differentiation

coefficients OKT 4^+/OKT 8^+ and B-lymphocytes (Ig^+) in patients suffering from thymomegalia, thymectomia, infectious arthritis, furunculosis and mammary carcinoma (following radiation therapy). In the lymphocyte cultures of the peripheral blood of the patients, the percentage of subpopulations of lymphocytes was determined according to the immunofluorescence method with the aid of monoclonal antibodies, before and after introduction into the cell cultures of thymalin and the preparation of the invention in the most effective doses - 1μg/ml or 0.01μg/ml.

The test results are shown in Tables 5 and 6.

Table 5

Influence of thymalin on the subpopulations of lymphocytes in patients of different pathology (in the numerator - the initial number, in the denominator - after addition of thymalin)

Pathology	Number of tests	Number of Lymphocytes (%) X±m	
		T-helper cells (OKT 4^+)	T-suppressors (OKT 8^+)
1	2	3	4
Healthy	18	35.3±2.7 / 38.2±2.9	21.3±0.9 / 18.8±0.8
Thymomegalia	8	23.4±1.7 / 32.6±2.8^x	8.2±1.0 / 19.7±1.7^x
Thymectomia	4	10.5±1.3 / 20.6±1.7^x	14.5±0.7 / 16.1±1.1
Infectious arthritis	4	20.8±1.4 / 29.5±1.7^x	26.5±2.1 / 22.3±1.7
Furunculosis	6	16.6±1.2 / 26.9±1.1^x	12.0±0.9 / 18.3±1.3^x
Mammary carcinoma (after radiation therapy)	10	23.3±2.1 / 32.6±3.4^x	21.3±1.7 / 19.6+1.6

Continuation of Table 5

Pathology	Number of tests	Number of lymphocytes (%) X ± m	
		$\frac{\text{OKT } 4^+}{\text{OKT } 8^+}$	B-lymphocytes (Ig^+)
1	2	5	6
Healthy	18	$\frac{1.66 \pm 0.13}{2.03 \pm 0.16^x}$	$\frac{13.8 \pm 1.2}{12.1 \pm 1.1}$
Thymomegalia	8	$\frac{2.85 \pm 0.19}{1.65 \pm 0.11^x}$	$\frac{16.4 \pm 1.3}{11.4 \pm 1.1}$
Thymectomia	4	$\frac{0.72 \pm 0.08}{1.28 \pm 0.11^x}$	$\frac{12.5 \pm 1.0}{17.7 \pm 1.3^x}$
Infectious arthritis	4	$\frac{0.78 \pm 0.09}{1.32 \pm 0.11^x}$	$\frac{8.9 \pm 0.9}{14.3 \pm 1.2}$
Furunculosis	6	$\frac{1.38 \pm 0.82}{1.47 \pm 1.2^x}$	$\frac{19.9 \pm 1.7}{15.6 \pm 1.2}$
Mammary carcinoma (after radiation)	10	$\frac{1.09 \pm 0.04}{1.65 \pm 0.09^x}$	$\frac{15.6 \pm 1.2}{13.6 \pm 1.0}$

x - statistically significant ($P < 0.05$) in comparison with the initial data

Table 6.

Influence of the medicament of the invention on the subpopulations of lymphocytes in patients of different pathology (in the numerator - the initial number, in the denominator - following the addition of the medicament of the invention)

Pathology	Number of tests	Number of lymphocytes (%), X±m	
		T-helper cells (OKT 4^+)	T-suppressors (OKT 8^+)
1	2	3	4
Healthy	18	$\frac{35.4\pm2.7}{37.6\pm2.3}$	$\frac{21.3\pm0.9}{19.4\pm0.9}$
Thymomegalia	8	$\frac{23.4\pm1.7}{33.4\pm2.6^x}$	$\frac{8.2\pm1.0}{18.3\pm1.6^x}$
Thymectomia	4	$\frac{10.5\pm1.3}{21.1\pm1.5^x}$	$\frac{14.6\pm0.7}{15.3\pm1.3}$
Infectious arthritis	4	$\frac{20.8\pm1.4}{30.1\pm1.9^x}$	$\frac{26.5\pm2.1}{24.7\pm2.0}$
Furunculosis	6	$\frac{16.6\pm1.2}{27.4\pm1.2^x}$	$\frac{12.0\pm0.9}{19.1\pm1.4^x}$
Mammary carcinoma (after 10 radiation therapy)	10	$\frac{23.3\pm2.1}{31.7\pm2.9^x}$	$\frac{21.3\pm1.7}{18.6\pm1.9}$

Continuation of Table 6

Pathology	Number of tests	Number of lymphocytes (%), X±m	
		$\frac{OKT\ 4^+}{OKT\ 8^+}$	B-lymphocytes (Ig^+)
1	2	5	6
Healthy	18	$\frac{1.66\pm0.13}{1.94\pm0.11}$	$\frac{13.8\pm1.2}{12.6\pm1.3}$
Thymomegalia	8	$\frac{2.85\pm0.19}{1.83\pm0.12^x}$	$\frac{16.4\pm1.3}{12.6\pm1.3^x}$
Thymectomia	4	$\frac{0.72\pm0.08}{1.38\pm0.09^x}$	$\frac{12.5\pm1.0}{16.4\pm1.2^x}$
Infectious arthritis	4	$\frac{0.78\pm0.09}{1.22\pm0.12^x}$	$\frac{8.9\pm0.9}{14.1\pm1.3}$
Furunculosis	6	$\frac{1.38\pm0.82}{1.43\pm1.3^x}$	$\frac{19.9\pm1.7}{16.4\pm1.7}$
Mammary carcinoma (after radiation therapy)	10	$\frac{1.09\pm0.04}{1.70\pm1.5}$	$\frac{15.5\pm1.2}{14.7\pm1.2}$

x - statistically significant ($P < 0.05$) in comparison with the initial data

It was established that thymalin and the medicament of the invention in a dose around 100 times lower than that of thymalin normalises the ratio between the subpopulations of lymphocytes in all groups of the tested patients.

Thus, the results of the experimental examination of the new medicament demonstrated its high effectiveness in the treatment of immunodeficient conditions. The medicament has a strong effect (around 50 to 500 times stronger than that of thymalin) on the immunological reactivity of the organism. Under the influence of the

medicament, a substantial stimulation of the production of lymphocytes and T-lymphocytes and a marked modulation of the ratio between the subpopulations of the immuno-competent cells is detected.

The medicament of the invention can be used in different galenical forms: in the form of injection solutions, tablets, suppositories, ointments. Where the medicament of the invention is used in the form of injection solutions, the solutions are used with a content of active substance of 0.001 to 0.01 % by weight (0.0001 to 0.001 mg/kg body mass or 10 to 100 mg active substance per 1 ml solvent). As diluent, the medicament preferably contains 0.9% aqueous sodium chloride solution, distilled water, Novocaine solution for the injections, Ringer's solution or glucose solution.

When in the form of tablets and suppositories, the medicament preferably contains the active substance in a quantity of 0.1 mg per one table or one suppository. For the tablets and suppositories, any pharmaceutically suitable base is used as the pharmaceutical filling agent. When the medicament in the form of an injection solution is introduced intramuscularly, one injection is introduced within 5 days. When the medicament is introduced intranasally, one ampoule or one tube of ointment is also introduced within 5 days.

The medicament of the invention does not exhibit any secondary effect and does not have any contraindications. The galenical forms of the medicament of the invention are produced according to the generally known method.

The medicament of the invention is used in medicinal practice for the treatment and prophylaxis of human immunodeficient conditions.

Claims

1. A medicament for the treatment of immunodeficient conditions, which provides an active substance of peptide nature and a pharmaceutical diluent, characterised in that, as the active substance of peptide nature, it contains a peptide of the following structure H-L-Glu-L-Trp-OH.

2. A medicament according to Claim 1, in the form of an injection solution, characterised in that it contains the active substance in a quantity of 0.001 to 0.01 % by weight.

3. A medicament according to Claims 1 to 2, characterised in that, as the pharmaceutical diluent, it contains a 0.9% aqueous sodium chloride solution and/or a 0.5% aqueous Novocaine solution.

4. A medicament according to Claim 1 in the form of tablets, suppositories or capsules, characterised in that it contains the active substance in a quantity of 0.1 mg per one tablet, suppository or capsule.

5. A medicament according to Claim 4, characterised in that, as the pharmaceutical diluent, it contains the filling material -- starch, glucose, glycine*.

*Footnote: see footnote on page 2.

* C L M S *