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(54) Titre : UTILISATION D'UN DIPEPTIDE POUR STIMULATION DE PROCESSUS DE REGENERATION
(54) Title: USE OF A DIPEPTIDE FOR STIMULATING REPAIR PROCESSES

(57) Abrégé/Abstract:
L-Lys-L-Glu dipeptide is proposed for use in medicine for preparation of a drug capable of stimulating processes. According to the invention, the pharmaceutical peptide preparation capable of stimulating regeneration consists of pharmaceutically admissible carrier and effective quantity of dipeptides as an active part, which is a combination of. L-lysil-L- gluthmine acid or its salts. The pharmaceutical peptide preparation is proposed for the parental, intranasal, oral and local application. According to the invention, the method stimulating regeneration consists of prophylactic and/or treatment injections of the drug in the dose of 0,0.1-100 μg per kg of weight, at least once a day during a period necessary for obtaining a therapeutic effect.

Abstract

L-Lys-L-Glu dipeptide is proposed for use in medicine for preparation of a drug capable of stimulating processes. According to the invention, the pharmaceutical peptide preparation capable of stimulating regeneration consists of pharmaceutically admissible carrier and effective quantity of dipeptides as an active part, which is a combination of: L-lysil-L-gluthmine acid or its salts. The pharmaceutical peptide preparation is proposed for the parental, intranasal, oral and local application. According to the invention, the method stimulating regeneration consists of prophylactic and/or treatment injections of the drug in the dose of 0,0.1-100 μg per kg of weight, at least once a day during a period necessary for obtaining a therapeutic effect.

USE OF A DIPEPTIDE FOR STIMULATING REPAIR PROCESSES

Field of Invention

The invention is referred to pharmacology, i.e. pharmaceuticals containing peptides and their compositions, which may find prophylactic and/or therapeutic employment in medicine as stimulators of tissue regeneration in case of pyo-inflammatory diseases and post-operative complications, trophic disorders, diseases and injuries of skin and mucous membrane, consequences of radiation, thermal and chemical factors accompanied by the disturbances of repair processes.

The invention is concerned with application of dipeptide L-lysil-L-gluthamine acid (L-Lys-L-Glu) as a substance stimulating repair processes in subjects, which need it.

Background of the Invention

Among the drugs most analogous in application to the invented one there is a group of preparations stimulating metabolic processes: derivatives of pirimidine (Methyliracil, Pentoxyl) and biogenic preparations (Actovegin, Solcoseryl) (1).

Methyluracil drawback consists in cutaneous allergic reactions (urticaria eruption), sometimes headaches and dizziness. Pentoxyl oral application may induce dyspepsia due to irritating action of the drug. The detriment of Actovegin and Solcoseryl consists in a small amount of active substances in the drugs, prolonged treatment and limited application with respect to the stage of the wound process, as well as low efficacy in treatment of purulent wounds. These drugs produce largely stimulating effect on leukopoiesis.

There is known dipeptide L-Lys-L-Glu used as a component for peptide synthesis (2).

It is well known that L-Lys-L-Glu dipeptide reveals immunomodulating activity (3). However, this activity of the dipeptide characterizes only the direction of its immunobiological action, which is not an obvious and interrelated manifestation of the dipeptide properties to stimulate repair processes, and does not specify indications for its clinical application. Below given examples of dipeptide L-Lys-L-Glu stimulating action on repair processes confirm objectively the absence of correlation between the known property and the claimed one.

Disclosure of the Invention

The claimed invention is aimed at the solution of problem of obtaining the substance of peptide origin capable to stimulate repair processes.

With respect to the invention, it is proposed to use dipeptide with the following amino acid sequence: L-Lys-L-Glu, as a substance revealing a property to stimulate repair processes.

Dipeptide is obtained by a classical method of peptide synthesis in a solution (4).

Previously unknown property of L-Lys-L-Glu dipeptide to stimulate repair processes was found out in its experimental study.

With respect to the invention there is proposed a pharmaceutical peptide preparation able to stimulate regeneration which contains pharmaceutically admissible carrier and effective amount of dipeptide as an active basis representing a compound L-lysil-L-gluthamine acid (L-Lys-L-Glu) or its chemical modification as salts.

With respect to the invention, pharmaceutical peptide preparation, stimulating repair processes, may contain salts according to amino group (acetate, hydrochloride, oxalate), or according to carboxyl groups (salts of metals – Natrium, Calcium, Lithium, Zinc, Magnesium and other organic and non-organic cations, for example ammonium, triethylammonium.

The notion "pharmaceutical peptide preparation", used in this application, implies the employment of any drug form, containing various pharmaceutical derivatives of the dipeptide, which reveal therapeutic effect in treatment of the diseases requiring stimulation of tissue regeneration.

The notion "effective amount", used in this application, implies the employment of such an amount of the active base, which in compliance with its quantitative indices of activity and toxicity, as well as with respect to the knowledge available, must be effective in this drug form. In order to obtain pharmaceutical compositions meeting the invention, the proposed dipeptide or its pharmaceutically applicable derivatives are blended as an active ingredient and a pharmaceutical carrier in accordance with accepted in pharmacology methods of compounding.

The carrier may have various forms, which depend on the drug form of the preparation, desirable for administration, for example: parenteral, oral, intranasal or local (e.g. as applications or ointments).

All known pharmaceutical components may be used for preparation of compositions in preferable doses for oral or local application.

For parenteral (intranasal) administration, the carrier usually includes sterile water, although there could be used other ingredients instrumental for stability or maintaining sterility.

In accordance with the invention, the dipeptide is active in case of its administration in doses 0.01-100 μg/kg of the body weight, although there could be used lower (higher) doses with respect to severity and character of the disease.

The claimed pharmaceutical peptide preparation is proposed for parenteral, intranasal, oral and local application.

The invention embraces both method of stimulation of the regeneration processes for humans and animals who need such stimulation and pharmaceutical compositions for implementation of this method.

In accordance with the invention, the method of stimulation of the processes of regeneration by means of administration of the drug containing, as an active base, dipeptide L-lysil-L-gluthamine acid (L-Lys-L-Glu) or its chemical modifications in the form of salts or other derivatives, is manifested in the activation of cell metabolism and regulatory effect on the processes of proliferation and differentiation of cells of various tissues. The method includes prophylactic or therapeutic exposure of the subject, which needs it, to the drug in doses 0.01-100 μg/kg of the body weight at least once a day during the period necessary for reaching therapeutic effect – 10-40 days with respect to the character and severity of the disease.

The invention involves prophylaxis and treatment of the diseases, which require stimulation of tissue regeneration: pyo-inflammatory processes and post-surgical complications, trophic disturbances, skin and mucous diseases and lesions, aftereffects of radiation, thermal, and chemical factors, accompanied with the shifts of repair processes.

Industrial Application

The invention is illustrated by the examples of synthesis of the dipeptide with the formula L-lysil-L-gluthamine acid (L-Lys-L-Glu) (example 1), those of the tests for toxicity and biological activity of the dipeptide (examples 2, 3, 4 and 5) and examples of the results of clinical application of the dipeptide, demonstrating its pharmacological properties and confirming the possibility of reaching therapeutic effect (examples 6, 7, 8). Reference is also made to the drawings.

Brief Description of the Drawings

The foregoing and other objects, aspects and advantages of an exemplary embodiment of the invention will next be described in greater detail, with the aid of a drawing. Shown are:

FIG. 1A shows a PCNA-positive nuclei of proliferating cells in the generative crypt zone of the duodenum. Avidin-biotin-peroxydase method–×100. Irradiated animal.

FIG. 1B shows a PCNA-positive nuclei of proliferating cells in the generative zone of the duodenum. Avidin-biotine-peroxydase method–×100. Irradiated animals +L-Lys-Glu dipeptide.

FIG. 2A demonstrates a serotonin-immunopositive cells in the mucous membrane of the duodenum. Streptavidin-biotin-peroxydase method–×100. Irradiated animals.

FIG. 2B shows a serotonin-immunopositive cells in the mucous membrane of the duodenum. Streptavidin-biotin-peroxydase method–×100. Irradiated animals +L-Lys-L-Glu dipeptide.

FIG. 3A shows a metallotionein-immunopositive cells in the mucous membrane of the duodenum (histotopographic localization of MLT-positive cells). Streptavidin-biotin-peroxydase method–×100. Irradiated animals.

FIG. 3B shows a metallotionein-immunopositive cells in the mucous membrane of the duodenum (histotopographic localization of MLT-positive cells). Streptavidin-biotin-peroxydase method–×400. Irradiated animals +L-Lys-L-Glu dipeptide.

FIG. 4A shows a mast cells in the duodenum mucous membrane. Selective staining with toluidin blue, pH 0.5–×100. Irradiated animals.

FIG. 4B shows a mast cells in the duodenum mucous membrane. Selective staining with toluidin blue, pH 0.5–×100. Irradiated animals +L-Lys-L-Glu dipeptide.

Example I. Synthesis of L-Lys-L-Glu dipeptide

1. N*a*, Nε – dibenzyloxycarbonyllizyl – γ – benzylgluthamine acid [I].

0.154 g (0.65 mmol) of γ - benzylgluthamine acid are suspended in 3 ml of dimethylphormamide and added 0.091 ml (0.65 mmol) of triethylamine while mixing, then 0.300 g. (0.59 mmol) of N-

oxysuccinimide ether of N*a*, Nε – dibenzyloxycarbonyllizyl. Reacted mixture is blended within 12 hours at room temperature. Afterwards the solvent is boiled down in the vacuum under 40°C, and 10 ml of 1n H_2SO_4 are added to the residue. The product is twice extracted by the ethyl acetate (30x2). The organic layer is bathed in 1n H_2SO_4 and water up to neutral reaction and dried over Na_2SO_4. Solvent distillation is conducted under vacuum at 40°C and the residue is dissolved in 1-2 ml of ethyl acetate. The product is set down by hexane and recrystallized in the system ethyl acetate/hexane. The product is filtered and dried under vacuum over P_2O_5. The yield is 0.0330 g (88%). The coefficient of retaining R*f* = 0.81 (benzol : acetone is 1 : 1, silufol).

2. L- Lysil-L-Gluthamine acid.

Defended dipeptide [I] (0.330 g) is dissolved in 10 mg of methanol, added with 3 ml of water and hydrate over palladium on coal. The control is conducted by thin layer chromatography. Upon the completion of hydration the catalyst is filtered out and the residue is dissolved in minimal quantity of water and set out by methanol. The product is filtered, bathed in ethanol, dried under vacuum over P_2O_5. The yield is 0.110 g (85%). Temperature of melting being 194 - 196°C. $[\alpha]\ \gamma^{20}$ = +20.0° (c = 3.0; H_2O). R*f* = 0.54 (acetonyl:water 1:3, "Merk"). Electrophoresis: E_{Gly} = 1.96; E_{his}= 0.98 (1400 volt, 45 min., 2% acetic acid, "Watmann 3MM".

To obtain corresponding salts according to carboxyl group, free dipeptide is added calculated amount of water solution of hydroxide of the corresponding metal (NaOH, KOH, $ZnOH_2$, LiOH, $CaOH_2$, $MgOH_2$, NH_4OH). To obtain triethylammonium salt, the processing is conducted in the same way using triethylamine as a base.

Example 2. Study of L-Lys-L-Glu dipeptide for toxicity.

The study of general toxic activity of dipeptide L-Lys-L-Glu was conducted in compliance with "The rules of pre-clinical estimation of safety of pharmacological substances (GLP)".

The purpose of study consisted in the identification of tolerable toxic doses of the drug, estimation of stage and character of pathological alterations in various organs and systems of the organism and determination of correlation between toxic effect related to dose and duration of drug application.

The estimation of acute toxicity of dipeptide L-Lys-L-Glu was conducted according to Kerber. The study was carried out on 66 white outbred male mice with body weight 20-23 g, kept under standard regimen and fed with standard chow in the vivarium. The animals were divided at random into 6 equal groups by 11 mice in each. The animals were exposed to single administration

of the drug intramuscularly, 0.25 ml in doses 1 mg/kg, 2 mg/kg, 3 mg/kg, 4 mg/kg, 5 mg/kg , several thousand times exceeding therapeutic dose recommended for clinical trial. Control animals were administered with sodium chloride solution in the same amount.

In 72 hours and later on in 14 days, none of the animals in either of the groups died. No changes in the general state, behaviour, locomotor activity, hairy or skin integument or physiological discharges were registered.

Thus, L-Lys-L-Glu dipeptide in doses several thousand times exceeding the therapeutic one, recommended for clinical trials does not induce any toxic effects, which points out wide therapeutic applicability of the drug.

The study of sub-acute toxicity of L-Lys-L-Glu dipeptide was carried out on 60 white outbred mice with body weight 150-250 mg. The animals of experimental groups were exposed daily to single administration of the drug intramuscularly for 90 days in doses 1 mg/kg, 0.3 mg/kg, 3 mg/kg in 0.5 ml of sodium chloride solution. The animals of control groups were administered with sodium chloride solution in the same quantity.

During the whole period of investigation the animals were under daily control. There was monitored animal behaviour, as well as chow and water consumption, condition of hairy and mucous surfaces. The animals were weighed weekly. Morphological composition and properties of peripheral blood were studied prior to and on the 30^{th}, 60^{th} and 90^{th} day of drug administration. Biochemical and coagulologic indices of the blood were studied upon experiment completion.

Chronic toxicity of L-Lys-L-Glu dipeptide obtained by the claimed method was studied during its longitudinal administration to rats with body weight 150-250 mg. The animals of experimental groups were exposed daily to single intramuscular administration of the substance in doses 1 mg/kg, 0.3 mg/kg, 3 mg/kg in 0.5 ml of sodium chloride solution for 6 months. There was analyzed animal behaviour, as well as chow and water consumption, condition of hairy and mucous surfaces. The animals were weighed daily during the first 3 months of the experiment and then once a month. In 3 months after the onset of administration and upon completion of the experiment there were conducted hematological and biochemical examinations. There were estimated functions of cardio-vascular system, liver, pancreas, kidney and adrenal gland. Upon the termination of the drug administration some animals were exposed to pathomorphological examination with the purpose of studying the state of various departments of the brain and spinal marrow, heart, aorta, lungs, liver, organs of endocrine and immune systems.

The estimation of general state of animals, morphological and biochemical indices of peripheral blood, morphological state of intrinsic organs, cardio-vascular and respiratory systems, liver and kidney functions revealed no pathological changes in the organism.

The study of sub-acute and chronic toxicity of L-Lys-L-Glu dipeptide evidence the absence of side effects during long-term application of the drug in doses exceeding therapeutic one 100-1000 times.

Example 3. Influence of L-Lys-Glu dipeptide on the healing of purulent incised crushed wounds of the soft tissues

The efficacy of L-Lys-L-Glu dipeptide was estimated on the model of purulent incised crushed wounds of the femural soft tissues in "Shinshilla" rabbits of both sexes with body weight 2-3 kg. Therefore, the rabbits underwent shaving in the area of femural soft tissues with subsequent incision 5 cm long and 2 cm deep. Soft tissues (muscles, subcutaneous fat) were crushed with Kocher's forcepts and infected with the pathogenic mixture: Staphylococcus aureus, strain 186. Afterwards, the wound was sutured. In 72 hours the sutures were lifted, and the wound was treated with 3% solution of hydrogen peroxide.

The animals of experimental group were injected intramuscularly every day for five days with dipeptide L-Lys-L-Glu in a single dose 1 μg/kg per injection. Control rabbits were injected with sodium chloride solution according to the same scheme. In the process of treatment the wounds were treated with antiseptic remedies for external use.

The estimation of L-Lys-L-Glu dipeptide efficacy in the dynamics of the inflammatory process regression was based on the terms of crust rejection and wound clearance from the pyo-necrotic mass, appearance of the granular tissue in the wound and beginning of edge epithelization. With the purpose of identifying objective criteria for the course of the wound process there were analyzed indices reflecting semi-quantitative characteristics of separate cellular elements and structures of the granular tissue on days 6, 14, 21, 28 and 40. There was also estimated the activity of tissue enzymes (5,6,7).

As a result of investigation it was registered that animals of all groups in the first phase of inflammation revealed on the 6^{th} day extended necrosis in the tissues encircled with a thin rim of the granular tissue with diffused fresh fibroblasts and single histiocytes. In the phase of proliferation, small foci of necrosis were encircled with a wide layer of the granular tissue with an abundance of vessels and lymph cells. The amount of histiocytes increased and macrophages formed clusters in necrotic zones. Fibroblasts were stretched and had thin nuclei. Especially pronounced were processes of cellular elements activation in animals of experimental group (Table 1). In the phase of cicatrization these animals showed foci of necrosis encircled with the layer of the granular tissue with mature fibroblasts. Between fibroblasts there was noted the layer of collagenous fibres. Interstitial substance close to necrotic zones contained pre-collagenous fibres,

fibroblasts, histiocytes suggesting the process of resorption and substitution of necrotic tissues with fresh granular tissue.

A prominent feature of tissue response to the application of L-Lys-L-Glu dipeptide consisted in high activity of acid phosphatase in histiocytes in the phase of proliferation (14-28 day). In few foci of leukocytory infiltration, as well as in the vascular endothelium, there was noted a high activity of alkaline phophotase. In the phase of cicatrization, a high content of acid phosphatase stabilized in histiocytes, and that of alkaline phosphatase – in leukocytes and vessels (Table 2).

The changes observed evidence intensification of cell metabolism in tissues, promoting fast clearance of the wound surface from necrotic tissues with subsequent wound epithelization (Table 3).

Example 4. L-Lys-L-Glu dipeptide's influence on the compensatory regeneration of the liver after its partial hepatectomy

The research has been conducted on 26 white outbred male rats weighing 150-200 grams. The animals were divided into thee following groups:

1 group – healthy animals;

2 group – control (rats which had undergone partial hepatectomy, with 2/3 of their liver ectomized);

3 group- rats which had undergone the operation and were subsequently (2 and 24 hours after the operation) made two subcutaneous injections of L-Lys-L-Glu dipeptide (0,1 μg/kg per rat).

At the same time, the animals of the first and second groups were made injections of the same volume of the sodium hydrochloride. The extracted liver was fixed in formalin.

The rats, which had been operated on, were killed by means of ether 32 and 96 hours after the operation. The rats from the control group were killed too, simultaneously. Their livers were fixed in formalin. After staining the preparations with hematoxilin-eosin, the mitotic index in the liver cells was defined, as well as the quantity of polyploid cells in the S-phase cell cycle (the quantity of dividing cells).

The study of mitotic activity of the regenerating liver cells 32 hours after a partial hepatectomy showed that the number of mitoses and cells in the S-phase of the cell cycle becomes two times bigger than in the liver of healthy animals. These differences are not reliable in the case of injecting the sodium hydrochloride, while after the L-Lys-L-Glu dipeptide injections, the increasing number of mitoses, DNA- synthesizing cells and the overall amount of dividing cells does become trustworthy.

The study of the liver preparations 96 hours after the hepatectomy proved that both, rats receiving the sodium hydrochloride and those injected with L-Lys-L-Glu dipeptide, demonstrated considerable intensification of mitotic activity of hepatocytes. Comparing the data of the third and second groups, it became clear that the rats injected with L-Lys-L-Glu dipeptide had the number of mitoses twice as big as the rats injected with the sodium hydrochloride. The number of cells in the S-phase of their mitotic cycle of the third group of rats did not differ reliably from the number of hepatocytes in the S-phase of the second group, though, on the whole, the number of dividing cells, 96 hours after the hepatectomy in the regenerating liver of the rats injected with L-Lys-L-Glu dipeptide, was 75% bigger than with rats injected with the sodium hydrochloride (table 4).

Thus, it has been proved that the rats, injected with L-Lys-L-Glu dipeptide 96 hours after the partial hepatectomy, demonstrated intensification of the mitotic activity of hepatocytes, testifying to the acceleration of reparative processes in the liver.

Example 5. Influence of L-Lys-L-Glu dipeptide on regeneration of the intestinal mucous membrane after the radiation injury

The work has been performed on 24 two-month-old white male rats of the Wistar line, weighing 90-100 grams. The following three groups of animals were researched:

1 group - healthy animals;

2 group – control (irradiated animals);

3 group – irradiated animals injected with L-Lys-L-Glu dipeptide.

A general single γ- irradiation of 6 Gy, inducing "the syndrome of intestinal death", was made by the cobalt apparatus GUB 20000, with the dose capacity of 200 rad/min.

L-Lys-L-Glu dipeptide was injected 24 hours after the irradiation – 0.5 μg/kg in 0,5 ml of the sodium hydrochloride – intraperitoneally during 5 days. The animals from the first and second groups received the sodium hydrochloride by the same scheme.

The study of L-Lys-L-Glu dipeptide's action has been performed on the proximal section of the duodenum of irradiated animals.

The animals were killed under nembutal narcosis (50 mg/kg) on the 8^{th} day after irradiation (beginning of the period of repair regeneration). Pieces of the duodenum were being fixed during 24 hours by Karnovsky for the electronic microscopy.

The ultra-structural research was conducted under the JEM-100S microscope (JEOL, Japan) at the ultra-thin microscopic sections prepared on the LKB-7A ultra-microtome (LKB, Sweden) and contrasted by uranilacetate and lead cytrate.

Mast cells were selectively stained with 1 % solution of toluidine blue (Fluka) in 0,5 M HCl, with pH- 0,5 (8,9).

To study the proliferative activity of cells, were used mice's monoclonal antibodies to the proliferating cell nuclear antigen –PCNA- diluted at 1:50 (clone PC 10, Calbiochem, the USA), and avidin-biotin-peroxidase set for revealing mice's immunoglobulins (Vectastain, the USA).

Serotonin-positive cells were revealed with the help of polyclonal rabbits' antibodies to serotonin (Ready-to-Use) and streptavidin-biotin-peroxidase set (BioGenex, the USA). To reveal MTL-positive cells, were used rabbits' antibodies to metal-tioneins (1:2000).

Immunohistochemical identification of antigenes at the histological sections was made according to the main requirements to immune-peroxydase methods (10,11).

The quantity research was conducted with the help of the system of computer analysis of microscopic images – IMSTAR (Imstar, France) – the Morphostar-2 and Colquant-2 license applied software were employed, in accordance with the general principles of stereology in morphometry (12, 13). For each animal, the calculation of corresponding structures was made in ten visual eye sights by three sections of the studied organ. The mitotic index (I_{mit}) and the index of proliferating ability (I_{pcna}) of the duodenum cells were defined in 10-15 standard sections of crypts, with the overall contents of not less than 1000 nuclei of enterocytes. The test area for finding serotonin-positive and mast cells covered not less than 3 mm^2. MTL-positive cells were counted in 100 duodenum crypts.

Against the background of ionizing radiation, on the eighth day, a partial – in many cases almost total – restoration of enterocytes' ultra-structure is observed, however, there still occur hyperplasia (" swollen") mytochondria, edema of endoplasmic reticulum, and focal vacuolization of cytoplasm, endocrine cells looking practically unchanged by that time.

Quantity changes in the duodenum of the survived on the eighth day, after general instantaneous γ- irradiation of 6 Gy, have the following specific features: Ipcna in the intestinal crypts rises up to 46,5 %, while the mitotic index rises up to 4,2% (table 5). These data testify to the fact that restoration of the mucous epithelium of the survived animals goes very quickly, while the pool of trunk cells of the intestinal epithelium is in this period at the stage of hyper-regeneration (figure 1a).

Histological study of preparations stained with hematoxilin and eosin also testifies to the start of normalization processes of epithelial architectonics. Computer analysis results, though, show that numerical density of enterochromaffinn cells (figure 2a) and MTL-positive cells (figure 3a) does not reach yet the indices level of healthy animals.

The content of mast cells in the mucous plate proper of the irradiated animals is lowered by 10 times (figure 4a), which testifies to the uttermost radio-sensitivity of the mucous-type mast

cells to the ionizing radiation, as well as to a very slow restoration of their quantity, even when exposed to sublethal doses.

It's noteworthy that the injection of L-Lys-L-Glu dipeptide makes structures of the cytoplasmic reticulum and those of the plate complex in the duodenum endocrine cells more active, which testifies to its stimulating effect on synthesis processes and hormones secretions.

According to the results of the morphometric analysis, in the crypts of the irradiated animals' bowels, after the injection of L-Lys-L-Glu dipeptide, there occurs a considerable acceleration of restoration processes (figure 1b). Index PCNA reaches 49,8%, while the mitotic index increases up to 4,7% (table 5). The quantitative density of enterochromaffinn cells practically gets equal to that of healthy animals. There is a tendency to the increase of number and intensity of immune-staining of MTL-positive cells in the crypts' base (figure 3b).

Application of L-Lys-L-Glu dipeptide intensifies the proliferative potential of trunk cells of the bowels and enhances morpho-functional regeneration of the intestinal mucous after the general instantaneous γ-irradiation with dose of 6 Gy.

Thus, the experimental study proved that L-Lys-L-Glu dipeptide is not toxic, it activates metabolic processes and proliferative cell activity of any tissue enhancing their regeneration.

L-Lys-L-Glu dipeptide's features, revealed during the experimental pre-clinical study, allow to prescribe it as a prophylactic and/or treatment use as a stimulator of tissue regeneration for pyo-inflammatory diseases, post-operation complications, trophic disorders, skin & mucous injuries and diseases, radiation, thermal and chemical after-effects, accompanied by the disorder of repair processes.

The examples of the claimed dipeptide's clinical studies, presented below, demonstrate its pharmacological characteristics and confirm its patentability.

Example 6. Effectiveness of L-Lys-L-Glu dipeptide's use as a treatment for inflammation of salivary glands and sialolithic disease

45 patients were being observed. Of these, 27 people had inflammations of the salivary glands, 4 of whom had parotitis. 18 people suffered sialolithic disease of the submandibular gland. The average age of the patients was 35-40. All the patients with the sialolithic disease had the stones e0xtracted. 30 patients (15 of them had inflammation of the salivary gland and the other 15 had sialolithic disease) underwent daily intramuscular injections of L-Lys-L-Glu dipeptide 1 μg/kg for 5 days.

The patients of the control group underwent standard treatment: antibacterial, desensitization therapy, iodine-dimexid dressings, physical therapy (ultrasonics, 5-10% potassium

iodide electrotherapy on the gland area), local treatment (washing the glands with the solution of antiseptics and antibiotics).

The patients who had sialolithic disease and were treated with L-Lys-L-Glu dipeptide stopped having the discharge of pus from the gland ducts, in the post-operation period the wound in the mouth cavity was healed with the first intension, without any after-effects. Swelling and infiltration of soft tissues and mucous membrane of the mouth cavity were resolved on the third-fourth day after the operation. The gland reduced in size considerably, and pains stopped.

The patients who had inflammation of the salivary gland and were treated with L-Lys-L-Glu dipeptide, on the fourth-fifth day after the treatment, stopped having pains in the gland and the discharge of pus from the gland ducts, their salivation increased, the swelling and infiltration of the soft tissues were resolved; when palpated, the gland reduced in size considerably and became painless. The patients' general state improved. Their laboratory tests normalized as well.

Thus, the use of L-Lys-L-Glu dipeptide helped reduce the number of inflammations, facilitated regeneration of wounds, and shortened the period of treatment.

Example 7. Effectiveness of L-Lys-L-Glu dipeptide's use as a treatment for pyo-inflammatory diseases of different localization.

L-Lys-L-Glu dipeptide was used in the complex treatment of 15 patients who had flesh sluggish-granulating wounds in lower and upper extremities; and 19 patients who had phlegmon of the maxillofacial zone. 1 μg/kg of the preparation was injected intramuscularly, every day during ten days. Effectiveness of the treatment was evaluated in dynamics according to the changes in activity of wound enzymes and to the time of healing.

It was found that L-Lys-L-Glu dipeptide proved the most efficacious with the patients with low activity of wound proteoclastic enzymes in the first and second stages of wound process, with necrotic type of cytograms and slow healing. L-Lys-L-Glu dipeptide increased the activity of wound ferments in the first phase of the wound process, causing adaptation restructuring in the wound, and which empowered the synthesis of acid phosphatase in histiocytes, alkaline phosphatase in leukocytes and C cytochrome in macrophagi, intensifying the repair processes. Injecting the dipeptide helped to accelerate clearance of the wounds from necrotic tissues and to heal the wounds due to macrophagi, fibroblasts, and leukocytes activation in the inflammation nidus.

L-Lys-L-Glu dipeptide treatment resulted in the faster liquidation of a local inflammatory process, improvement of the general state of patients, and in reduction of the treatment period.

Example 8. Effectiveness of L-Lys-L-Glu dipeptide's use for cancer patients with post-operative complications.

L-Lys-L-Glu dipeptide was used in the complex treatment of 9 patients who had sluggish-granulating wounds after the surgical treatment for pulmonary cancer of the 2^{nd}-3^{rd} stages and cancer of the stomach of the 2^{nd}-3^{rd} stages.

In the pre-operation period, the patients underwent a radical-scheme radiation therapy, employing large fields of complex configuration on the linear electron accelerator (its power is 4,3 Mev) and gamma-therapy device "Rokus-M" in the brake mode. In particular cases, one of the components of the combined treatment was chemotherapy.

Beginning from the third day after the operation, 1 μg/kg of L-Lys-L-Glu dipeptide was injected intramuscularly daily during 10 days.

It was found that the use of the preparation helped to reduce edema and pain in the wound area, and accelerate both, clearance of the wounds from necrotic tissues and forming the post-operation cicatrix. During the period of injections, the following factors were observed: normalization of temperature, improvement of appetite and quicker weight gain.

Thus, the use of L-Lys-L-Glu dipeptide as a part of complex treatment of cancer patients stimulates repair processes in the tissues, promotes improvement of the general state of patients and reduces the time of their treatment.

Clinical application of L-lysil-L-gluthamine acid (L-Lys-L-Glu) confirmed the data obtained from experimental study that the preparation is an efficacious remedy against the disorder of repair processes.

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Table 1

Influence of L-Lys-Glu dipeptide on the dynamics of cellular elements and structures in rabbits with purulent incised crushed wounds of the soft tissues

Groups of animals	Terms of observaion (days)	Cellular elements and structures (conventional points)				
		Fibroblasts	Histiocytes	Leukocytes	Macrophages	Necrosis
Control	6	2	3	5	2	5
	14	3	3	5	2	4
	21	4	4	4	3	4
	28	4	4	2	3	2
	40	4	4	2	5	2
Dipeptide L-Lys-L-Glu	6	4*	4	5	3	3*
	14	5*	5*	5	3	2*
	21	5	5	4	2	2*
	28	5	4	4*	2	1
	40	6*	6*	2	2*	1

* - $P < 0,05$ as compared to the indices in animals of control group.

Notes:

1 point - absent;

2 points - single (traces);

3 points - few;

4 points - diffuse (moderately);

5 points - many;

6 points – very many.

Table 2

Influence of L-Lys-L-Glu dipeptide on the activity of tissue enzymes in rabbits with purulent incised crushed wounds of the soft tissues

Group of animals	Terms of observation (days)	Acid phosphotase in histiocytes (conv.points)	Alcaline phosphotase in leukocytes (conv.points)	Alcaline phosphotase in vessels (conv.points)
Control	6	3	3	2
	14	4	4	4
	21	4	4	3
	28	3	5	3
	40	3	5	2
Dipeptide L-Lys-L-Glu	6	5*	6*	6*
	14	6*	6*	5
	21	5	5	5*
	28	5*	6	4
	40	5*	5	4*

* - $P < 0,05$ as compared to the indices in animals of control group.

Notes:

1 point - absent;

2 points - single (traces);

3 points - few;

4 points - diffuse (moderately);

5 points - many;

6 points – very many.

Table 3

Influence of L-Lys-L-Glu dipeptide on the duration of the wound process in rabbits with purulent incised crushed wounds of the soft tissues.

Groups of animals	Terms of the onset of necrotizing tissue rejection from the wound surface (days)	Terms of complete clearance of the wound surface from necrotizing tissues (days)	Terms of the wound epithelization (days)
Control	12,9±1,2	21,1±3,2	31,8±1,7
L-Lys-L-Glu dipeptide	8,3±1,1*	14,2±1,3*	24,9±1,9*

* - $P < 0,05$ compared to the indices of control animals

Table 4

Influence of L-Lys-L-Glu dipeptide on the number of dividing cells in the rats' regenerating liver 32 and 96 hours after the partial hepatectomy (% of the total number of liver cells)

Groups of animals	Period of research		Mitotic index	% of cells in the phase of DNA synthesis	Total number of dividing cells
Healthy animals + sodium hydrochloride	—		0,682±0,013	1,752±0,463	3,403±0,498
Control (partial hepatectomy+ sodium hydrochloride)	32 hours	Before	0,431±0,019	1,043±0,127	1,474+0,143
		After	1,364±0,595	2,063±0,474	3,427±1,066
	96 hours	Before	0,417±0,053	0,924±0,091	1,342 ±0,060
		After	2,012±0,146*	3,417±0,295*	5,429±0,388*
Partial hepatectomy+ L-Lys-L-Glu dipeptide	32 hours	Before	0,450±0,067	0,870±0,100	1,320±0,159
		After	2,314±0,461**	3,882± 0,839**	6,196±1,279**
	96 hours	Before	0,294±0,084	0,982±0,141	1,276±0,128
		After	4,846±0,334*&	4,664±1,315**	9,510±1,609*#

* - P < 0,001 compared to pre-operation indices;

**- P < 0,05 compared to pre-operation indices:

& - P < 0,001 compared to the indices of the control group animals;

- P < 0, 05 compared to the indices of the control group animals.

Table 5

Influence of L-Lys-L-Glu dipeptide on the quantity characteristics of the main parameters of regeneration in rats' duodenum, based on the data of computer analysis of morphological images.

Groups of Animals	I_{mit}, %	I_{PCNA},%	ρ_{ser}, %	N_{MC}/1 mm^2	N_{MT}/100 crypts
Healthy Animals	2,9±0,1	44,8±0,2	0,64±0,02	169±23	132±8
Control (irradiated animals + physiological solution)	4,2±0,4*	46,5±0,7*	0,43±0,08*	18±2*	75±8*
Irradiated Animals + L-Lys-L-Glu Dipeptide	4,7±0,1*	49,8±0,7**	0,71±0,09	24±3*	102±9*

* – $P < 0,05$ in comparison to indices of the healthy animals;

** – $P < 0,05$ in comparison to the indices of the control group.

Notice:

Imit is the mitotic index (%) = Nmit /Ntotal x 100

Ipcna is the index of cell proliferative ability – PCNA (%) =Npcna /Ntotal x 100;

Pser is the volume density of serotonin-immunopositive cells

(an integral index of serotonin-immunopositive cells content in the tissue volume (%);

NMC / 1 square mm is the quantity of mast cells / 1 square mm;

NMT /100 crypts is the quantity of L-positive cells in 100 intestine crypts.

CLAIMS:

1. The use of L-lysyl-L-glutamic acid dipeptide (L-Lys-L-Glu) or a salt thereof for the prophylaxis and/or treatment of a disease requiring stimulation of tissue regeneration, wherein the disease belongs to any one of the following groups: pyo-inflammatory diseases and post-operative complications; trophic disorders; skin and mucous diseases and injuries; and after-effects of radiation, chemical and thermal factors.

2. The use of L-lysyl-L-glutamic acid dipeptide (L-Lys-L-Glu) or a salt thereof for the manufacture of a peptide preparation for prophylaxis and/or treatment of a disease requiring stimulation of tissue regeneration, wherein the disease belongs to any one of the following groups: pyo-inflammatory diseases and post-operative complications; trophic disorders; skin and mucous diseases and injuries; and after-effects of radiation, chemical and thermal factors.

3. The use of claim 1 or claim 2, wherein the dipeptide or salt thereof is used in admixture with a pharmaceutically admissible.

4. The use of claim 1, claim 2 or claim 3, wherein the dipeptide salt is a salt of an amino group of the dipeptide.

5. The use of claim 4, wherein the salts are selected from the acetate, hydrochloride and oxalate salts.

6. The use of any one of claims 1 to 5, wherein the dipeptide salt is a salt of one or more carboxyl groups of the dipeptide.

7. The use of claim 6, wherein the salts are salts of sodium, potassium, lithium, calcium, zinc, magnesium, or organic and non-organic cations.

8. The use of claim 6, wherein the salts are salts comprising ammonium or triethylammonium cations.

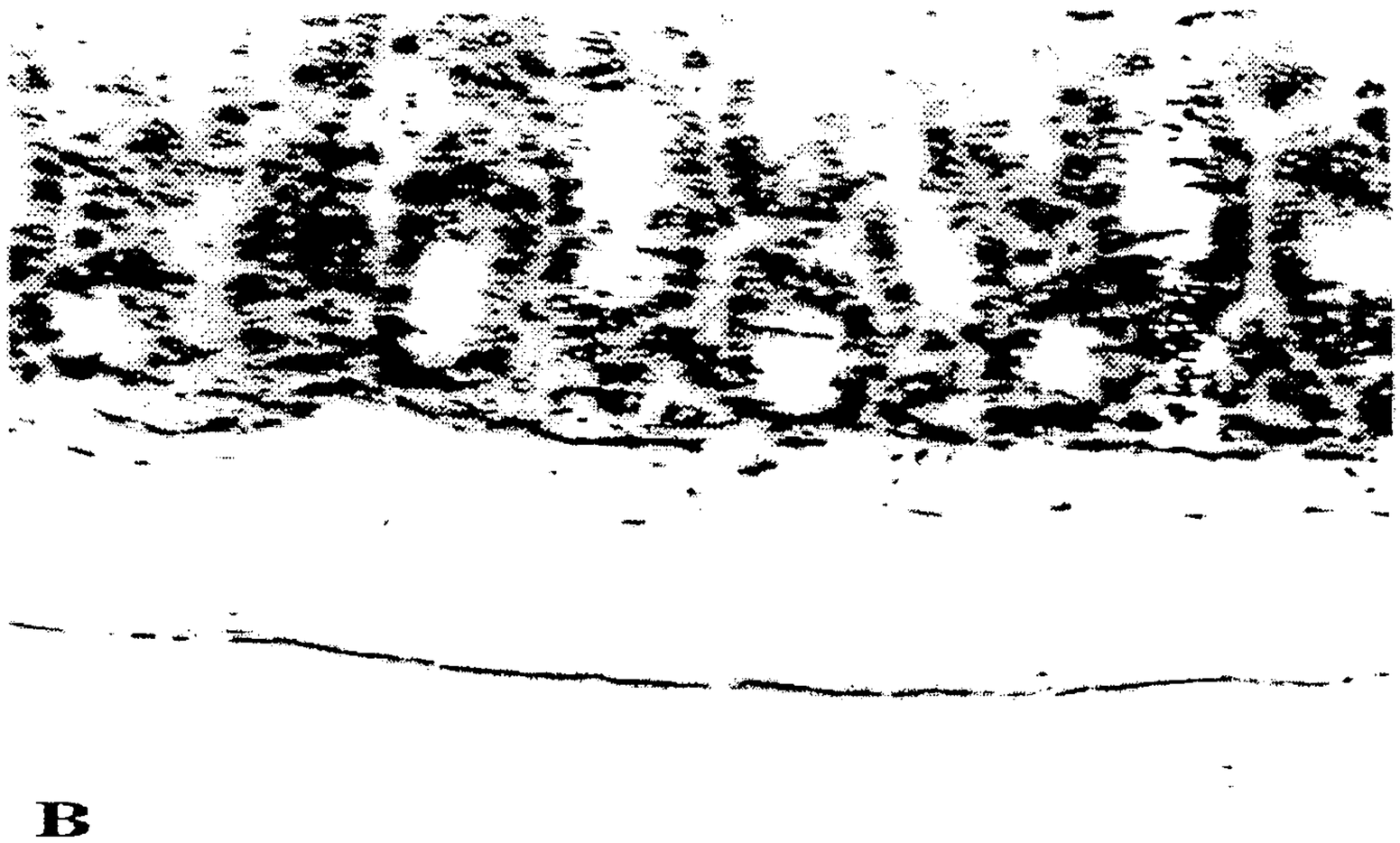
9. The use of any one of claims 1 to 8, wherein the dipeptide or salt thereof is in a form for parenteral, intranasal or oral administration or local application.

10. The use of any one of claims 1 to 9, wherein the unit dosage of the dipeptide or its salts is adjusted to 0.01 to 100 µg per kg bodyweight of a patient.

A

Figure 1 a.

PCNA-positive nuclei of proliferating cells in the generative crypt zone of the duodenum. Avidin-biotin-peroxydase method – x 100. Irradiated animals.



B

Figure 1 b.

PCNA-positive nuclei of proliferating cells in the generative crypt zone of the duodenum. Avidin-biotin-peroxydase method – x 100. Irradiated animals + L-Lys-L-Glu dipeptide.

Figure 2a.

Serotonin-immunopositive cells in the mucous membrane of the duodenum. Streptavidin-biotin-peroxydase method – x 100. Irradiated animals.

Figure 2b.

Serotonin-immunopositive cells in the mucous membrane of the duodenum. Streptavidin-biotin-peroxydase method – x 100. Irradiated animals + L-Lys- L-Glu dipeptide.

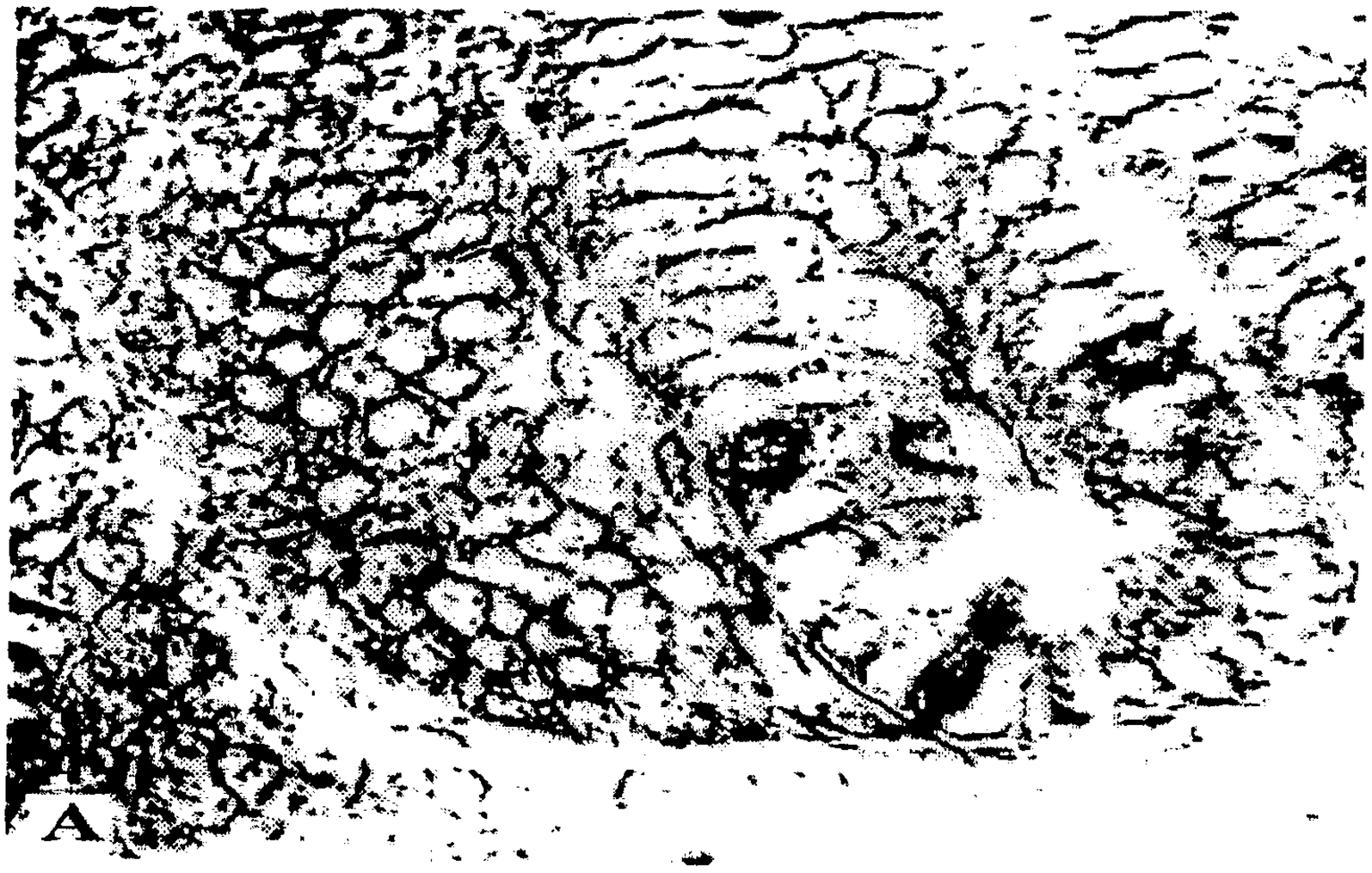


Figure 3a.

Metallotionein-immunopositive cells in the mucous membrane of the duodenum (histotopographic localization of MTL-positive cells). Streptavidin-biotin-peroxydase method – x 100 . Irradiated animals.

Figure 3b.

Metallotionein-immunopositive cells in the mucous membrane of the duodenum (histotopographic localization of MTL-positive cells). Streptavidin-biotin-peroxydase method– x 400. Irradiated animals + L-Lys- L-Glu dipeptide.

Figure 4a.

Mast cells in the duodenum mucous membrane.Selective staining with toluidin blue, pH 0,5 –x 100. Irradiated animals.

Figure 4b.

Mast cells in the duodenum mucous membrane.Selective staining with toluidin blue, pH 0,5 –x 100. Irradiated animals + L-Lys- L-Glu dipeptide.