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(71)(72) Applicant and Inventor: HENDRY, Neil, Geddes, Clarkson [GB/GB]; Tillyfoddie, Dunecht, Skene, Aberdeenshire AB3 7BB (GB).

(74) Agent: FITZPATRICKS; 4 West Regent Street, Glasgow G2 1RS (GB).

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(54) Title: TISSUE GROWTH REGULATION

(57) Abstract

A preparation for tissue growth regulation comprises (a) at least one of, an N-acetyl-D-glycosamine or an oligomer thereof or a deacetylated derivative thereof or a substituted product of these compounds, (b) at least one of biotin or an analogue or derivative of biotin or biologically active residue thereof, and (c) a divalent metal cation together with a pharmaceutically acceptable anion.

tissue repair, and are on the whole remote from the preparations to be disclosed hereinafter.

In patients who are malnourished, the systemic administration of amino acids is known to be of value in assisting wound healing, but only by remedying existing amino acid deficiencies.

Physical or physico-chemical techniques such as the use of magnetic fields or pulse electrical currents for localised stimulation of a wound site have some current vogue.

The inhibition of aberrant or malignant cells by chemical means is now common practice, and is effective where there is a favourable ratio of susceptibility between the malignant cells of the tumour and the normal cells of the host. In the absence of any more selective means of control, these cytotoxic substances have wide currency at present; but their toxicity and consequent high incidence of side effects makes it extremely desirable that improved physiological or chemical control should be developed.

Cortisone and its derivatives are effective in the control of excess fibrous tissue formation such as may occur in some types of injury, in many cases of burns, and after some inflammatory diseases of the joints. However, use of cortisone involves a considerable risk of side effects.

Earlier work (Nature, 199, 4991; 392; 1963) by the present inventor showed that the level of activity of the enzyme N-acetyl-D-glucosaminidase was closely related to what might be termed the "reactivity" of a variety of disease processes. This relationship existed under such a wide range of circumstances as to suggest a more important role than was then known for N-acetyl-D-glucosamine (NAG) and perhaps for other N-acetyl-D-glycosamines.

TISSUE GROWTH REGULATION

Field of the Invention

This invention relates to a preparation for tissue growth control in humans or animals. More particularly, the invention relates to a group of physiologically active preparations, at least one member of which promotes, whilst the others inhibit, the growth of both normal and abnormal tissue. Thus, the preparations of this invention provide for (a) the enhancement of normal tissue growth to accelerate normal repair functions and (b) the suppression of undesirable tissue growth, whether benign as in the fibrosis associated with excessive scar tissue formation, or aberrant, as in cancerous or other malignant change.

Background Art

Extensive work has been done on the control of growth in plants, none of which is applicable to animal tissue. Investigation of the growth requirements of cells of animal origin (including human cells) have been principally by tissue culture, that is by a technique facilitating the study of cells grown in isolation under laboratory conditions. Various "growth factors" have been used to encourage cellular proliferation, commonly using extracts of a relatively crude nature derived from whole organs such as mouse salivary gland or rat liver. Such growth factors have no possible applicability for systemic use in intact animals or humans. For example, riboflavin is thought to be a growth factor for rats, lipoic acid serves as a growth factor for certain micro-organisms and biotin is a growth factor for yeast and certain bacteria. Glycyl-histidyl-lysine and pituitary growth hormone are two chemically characterised pure substances with an influence on growth, the former in the culture of isolated liver cells and the latter in bodily growth as a whole. None of these "growth factors" has been recorded as having any known usefulness in

N-acetyl-D-glucosamine (2-acetamido-2-deoxy-D-glucose) residues are widely present in bound form in nature as is D-galactosamine.

In a text entitled "Processes in Pathology" (Taussig, M J) published in 1979 by Blackwell Scientific Publications, at pp 267-270, in a discussion primarily concerned with the activity of lectins, reference is made to NAG in a study of cell growth. Experiments relating to use of NAG are mentioned. In one of these NAG is used as its dimer di-N-acetylchitobiose bound to a protein carrier in an attempt to preimmunise mice against the lethal effects of a transplanted tumour with limited success. In another inhibition of tumour cell growth by treatment with monovalent lectin to cover the surface agglutination sites (NAG residues) was proved reversible by addition of excess of free NAG.

Other workers have been active in this field, for example, in the early 1960s Levvy and his associates at the Rowett Research Institute showed that the conversion to a lactone of a glycoside which formed the substrate for a glycosidase, would specifically inhibit the glycosidase concerned. Unfortunately, these lactones were stable only under strictly controlled laboratory conditions. Attempts to employ them for therapeutic purposes were therefore not pursued.

The inventor arrived at this invention by carrying out investigations into the possibility that N-acetyl-D-glycosamines and their oligomers and derivatives of both classes of compound might play important, and previously unrecognised parts in the processes of healing and repair, and in the invasiveness and proliferation of malignant cells. The concept of inhibition of the N-acetyl-D-glycosaminidases and of N-acetyl-D-glucosaminidase specifically, under physiological conditions, opens up new possibilities both for the

regulation of healing and repair, and for the control of cancerous growth.

Disclosure of the Invention

Accordingly, the present inventor provides a preparation for tissue growth regulation comprising (a) at least one of, an N-acetyl-D-glycosamine or an oligomer thereof or a deacetylated derivative thereof or a substituted product of these compounds, preferably with (b) at least one of biotin or an analogue or derivative of biotin or biologically active residue thereof, and (c) a divalent metal cation such as Mg, Ca or Mn together with a pharmaceutically acceptable anion.

Preferably, the component (a) of the preparation of this invention is N-acetyl-D-glucosamine or its oligomers or their completely or partially deacetylated derivatives.

Preferably, the preparation is prepared as an aqueous solution containing N-acetyl-D-glucosamine, biotin and a divalent cation such as Mg^{+2}. The solution may include buffer salts. As a solution, the preparation is suitable for parenteral or oral administration.

The preparation of this invention may be made in a solid form for oral or rectal administration by formulation of a tablet, lozenge or suppository. Alternatively, it may be made as a powder to be taken, or formulated as an encapsulated solution or emulsion for oral administration or subsequent formulation and administration as an injection solution. Further, it can be formulated for topical application in a suitable neutral ointment base.

The method of making the preparation affects the properties thereof. For example, mixtures may be activated by subjecting them to varying periods of, and conditions of, heating and of drying or partial drying, leading to the intermediate recovery of crystalline and associated insoluble materials, with other materials

remaining in solution or in a syrupy form. Those materials accompanying precipitated biotin on the one hand, and those materials remaining in solution after such precipitation and thereafter isolated in the syrupy or dried form on the other, have the separate biological activities referred to hereinbelow. Biological activity can also be obtained when the concentration of biotin and the periods and conditions of heating and drying or partial drying are so devised as to avoid the precipitation referred to above. Activity is then associated with the material either remaining in solution or produced in syrupy or dry form from it. These various materials influence the activity of N-acetyl-D-glucosaminidase and its two principal isozymes A and B. Depending upon the exact method of preparation, the materials may enhance or inhibit the whole enzyme or its A and B isozymes in isolation or together. The inhibition can be made to be either competitive or non-competitive. Thus, the preparation can influence the total level of N-acetyl-D-glucosaminidase activity in the body and also alter the proportional effect of the two main isoenzymes. In doing this, the preparation appears to mimic the reactions of the body to many disease processes as, for example, in its response to foreign tissue rejection, to inflammation and to malignancy.

For ease of understanding the invention, it will be described hereinbelow with reference to one glycosamine, N-acetyl-D-glucosamine, but it will be appreciated by workers in this field that the methods of preparation are applicable to analogous compounds and the biological effects obtained will vary depending on the enzyme or cellular system under investigation or therapy.

N-acetyl-D-glucosamine itself has been shown to have a stimulating effect upon defence cells in the body, and it is believed that the preparation of this invention

provides a new and selective means of influencing the body's immune mechanisms. The active components may be administered by mouth or by injection, either alone, or incorporated in physiologically-benign fluids or solids or any other pharmaceutically acceptable vehicles, carriers and adjuvants. Whilst not wishing to be bound by any particular theory, the role of the active components may be selectively to block or unblock active sites in enzymes participating in tissue or cell growth. There may be action on cell surface sites responsible for functions such as cell specificity, or the transfer of nutrients and hormones. Sites such as these are known to function abnormally in cancerous cells, and are affected also in viral and possible other infections.

Accordingly, the present invention finds a use in (a) the control of the proliferation, growth and invasiveness of malignant cells; (b) the regulation of the level of activity of cells involved in the repair of tissue after injury or inflammation, with the objective of encouraging healing whilst, at the same time, controlling any tendency for one aspect of healing to outstrip the other, as, for example, the over-production of collagen by fibroblasts results in hypertrophic scar formation, with consequent cosmetic or functional impairment; and (c) the stimulation of healing where it is indolent as, for instance, in the elderly or in those others having suffered very severe infections.

The invention will now be further described by way of the following Examples and Data indicating preferred methods of preparation and biological effects observed with use of the preparations of this invention.

Examples

Example 1 A method of preparing (a) a preparation for use in the stimulation of healing and (b) a preparation for the control of malignant cells.

(a) 3.23 g of N-acetyl-D-glucosamine, 200 mg of biotin, 375 mg potassium dihydrogen phosphate, and 1.8 g of magnesium sulphate were dissolved in water to give 100 ml of solution, which was maintained at 55°C in a closed vessel for 72 hours. The contents were then cooled and kept at just above freezing point. During the next 24 hours a mainly crystalline deposit separated. This was removed by centrifugation and the solid deposit was washed with water (or ethanol). The solid had no significant inhibitory power on N-acetyl-D-glucosaminidase, but had a powerful enhancing action on its B-iso-enzyme. The solid was insoluble in water, but soluble in lipids. Following treatment of patients with areas of indolent healing using the solid, rapid regeneration of skin cells was observed. Therefore, the aforementioned steps provide a means of stimulating healing.

(b) The supernatant remaining after separation of the solid material as described above was returned to a closed vessel and incubated. It was then freeze-dried. The resulting material showed a specific non-competitive type of inhibition of both the A and B isozymes of N-acetyl-D-glucosaminidase. When added to tissue cultures of malignant cells, such as Landschutz cells or Hela cells, it produced a series of dose-related effects, including the destruction of the cells' normal anti-adhesive mechanism, and the inhibition of growth. It also led to cell fragmentation and to the production of multiple non-viable sub-cellular fragments. When administered to patients with terminal malignancy, it controlled the invasiveness of the malignant process and increased survival, without other therapy, beyond the prognosis given by normal methods.

Example 2 A method of preparing a preparation for regulating the level of activity of cells involved in the repair of tissues following injury or inflammation.

3.2 g of N-acetyl-D-glucosamine, 10 mg of biotin, 375 mg of potassium dihydrogen phosphate and 1.8 g of magnesium sulphate were dissolved in water to provide 100 ml of solution which was placed in a closed vessel and maintained at a temperature of 70-80°C for 72 hours. The resulting solution was cooled and freeze-dried. the material resulting from this showed non-competitive specific inhibition of the A isozyme of N-acetyl-D-glucosaminidase and a varying degree of competitive inhibition only of the B isozyme. Addition of the material to cultures of fibroblasts produced a dose-related inhibition of fibroblast proliferation about 50% without causing cell death. When administered to competition horses with recent injuries, it produced diminution of swelling, and facilitated the return to soundness. In longer term therapy studies it has been administered on 47 occasions to 35 horses suffering mostly from injuries to the deep flexor tendon of the fore-limb, a condition frequently resulting in permanent disability, sometimes to a degree necessitating slaughter. All 35 horses returned to competition, usually at pre-injury level of soundness.

Systemic use in humans can be expected, but awaits further evidence of non-toxicity. However, topical application of the active substance in a neutral ointment base under the supervision of a consultant plastic surgeon has shown evidence of a capacity to control excessive scar tissue formation.

Claims

1. A preparation for tissue growth regulation comprising (a) at least one of, an N-acetyl-D-glycosamine or an oligomer thereof or a deacetylated derivative thereof or a substituted product of these compounds, (b) at least one of biotin or an analogue or derivative of biotin or biologically active residue thereof, and (c) a divalent metal cation together with a pharmaceutically acceptable anion.

2. A preparation according to claim 1 wherein the metal cation is selected from Mg, Ca, and Mn.

3. A preparation according to claim 1 or claim 2 wherein the glycosamine residue is that of D-glucosamine.

4. A preparation according to claim 2 wherein the cation is Mg^{2+}.

5. A pharmaceutical product comprising a preparation according to any one of the preceding claims in a physiologically-absorbable form together with one or more additives, as required, selected from a pharmaceutically acceptable vehicle, carrier, excipient, diluent or adjuvant.

6. A method of making a preparation for use in tissue growth regulation which comprises forming a solution of (a) at least one of, an N-acetyl-D-glycosamine or an oligomer thereof or a deacetylated derivative thereof or a substituted product of these compounds, (b) at least one of biotin or an analogue or derivative of biotin or biologically active residue thereof, and (c) a divalent metal cation together with a pharmaceutically acceptable anion, maintaining said solution at an elevated temperature for an extended period of time, and then freeze-drying the liquid to recover an agent for use in tissue growth regulation.

7. A method according to claim 6 wherein prior to freeze-drying the solution the method further comprises the

steps of cooling the solution to a temperature just above the freezing point of the solution to cause formation of a crystalline deposit, recovering this deposit for use as a further active agent for tissue growth regulation.

8. A method according to claim 7 wherein the liquid remaining after recovery of said deposit is incubated for a period of time prior to freeze-drying to recover an agent for use in tissue growth regulation.

9. A method according to claim 6 wherein the solution is heated to a temperature of from 50 to 60°C.

10. A method according to claim 6 wherein the solution is heated to a temperature of from 70 to 80°C.

11. A method according to any one of claims 6 to 10 wherein the heated solution is maintained at an elevated temperature for about three days.

12. A method according to claim 6 wherein the cooled solution is centrifuged to assist recovery of a crystalline deposit therein.

13. A method of making a preparation for tissue growth control according to either one of the Examples hereinbefore.

14. A method of regulating the growth of tissues which comprises introducing a preparation as claimed in any one of claims 1 to 5 to the locus of the tissues in order to contact tissue cells with the active components of said preparation.

15. A pharmaceutical composition for the treatment of indolent healing comprising a product derived by forming a solution of N-acetyl glucosamine, biotin, a soluble non-toxic salt of magnesium and a buffer, heating the solution to about 55°C and maintaining this temperature for about three days, cooling the solution and recovering a crystalline deposit therefrom for use in formulation of the composition.

16. A pharmaceutical composition for the treatment of

cell malignancy comprising a product derived by forming a solution of N-acetyl glucosamine, biotin, a soluble non-toxic salt of magnesium and a buffer, heating the solution to about 55°C and maintaining this temperature for about three days, cooling the solution and recovering a crystalline deposit therefrom, returning the solution to a closed vessel and incubating same for a period of time and thereafter freeze-drying same to recover material for use in formulating the composition.

17. A pharmaceutical composition for the treatment of injured or inflamed tissues comprising a product derived by forming a solution of N-acetyl glucosamine, biotin, a soluble non-toxic salt of magnesium and a buffer, heating the solution to a temperature of from 70 to 80°C and maintaining this temperature for about three days, cooling the solution and freeze-drying to recover material for use in formulating the composition.

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 86/00607

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[4]: A 61 K 33/06; A 61 K 31/70; // (A 61 K 33/06, 31:70; 31:415)

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[4]	A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
A	US, A, 3232836 (MICHAEL CARLOZZI) 1 February 1966, see column 3, lines 32-43; column 4, lines 9-38; claims 1-2	1-17

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* Special categories of cited documents: [10]

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
13th January 1987	06 FEB 1987
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	M. VAN MOL

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON -

INTERNATIONAL APPLICATION NO. PCT/GB 86/00607 (SA 14810)

This Annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 21/01/87

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US-A- 3232836		None	