

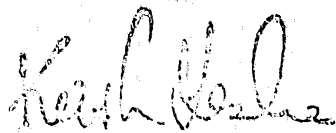
AUSTRALIA
PATENTS ACT 1990
NOTICE OF ENTITLEMENT

We, Per Prisell AND Gunnar Norstedt, the applicants in respect of Application No. 45253/89 state the following:-

The Nominated Persons are entitled to the grant of the patent because the Nominated Persons are the actual inventors.

The Nominated Persons are entitled to claim priority from the application listed in the declaration under Article 8 of the PCT because the Nominated Persons made the application listed in the declaration under Article 8 of the PCT, and because that application was the first application made in a Convention country in respect of the invention.

DATED this EIGHTH day of JULY 1991



.....
a member of the firm of
DAVIES & COLLISON for
and on behalf of the
applicant(s)

(D&C ref: 1407443)

(12) PATENT ABRIDGMENT (11) Document No. AU-B-45253/89
(19) AUSTRALIAN PATENT OFFICE (10) Acceptance No. 632074

- (54) Title
PHARMACEUTICAL PREPARATION
- International Patent Classification(s)
(51)⁵ **A61K 037/02 A61K 009/22 A61K 037/36 A61K 031/725**
- (21) Application No. **45253/89** (22) Application Date : **17.11.89**
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8804164 17.11.88 SE SWEDEN
- (43) Publication Date : **12.06.90**
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- (71) Applicant(s)
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- (56) Prior Art Documents
AU 17965/83
AU 61290/80
WO 88/07078
- (57) Claim

1. Pharmaceutical preparation for use in vivo to achieve slow release of growth factors and hormones, characterized in that it is a combination of: one or several receptor/binding proteins, optionally modified, ~~such as truncated~~, for binding growth factors or hormones, selected from the group, consisting of
Insulin-like growth factor-1-receptor;
Insulin-like growth factor-2-receptor;
Insulin-receptor;
Platelet derived growth factor-receptor;
Fibroblast growth factor-receptor;
Epidermal growth factor-receptor;
Nerve growth factor-receptor;
Colony stimulating factor-receptor;
Transforming growth factor-receptors;
Growth hormone-receptor;
Parathyroid hormone-receptor;
Calcitonin-receptor;
Estrogen-receptor;
Tumour necrosis factor-receptor;
Insulin-like growth factor serum binding protein;
Corticosteroid binding globulin;
and hyaluronic acid, or its derivatives, or a biodegradable polymer, selected from the group consisting of
Polyglycolide (PGA);
Copolymers of glycolide such as
Glycolide/L-lactide copolymers (PGA/PLLA)

(11) AU-B-45253/89

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(10) 632074

Glycolide/trimethylene carbonate copolymers (PGA/TMC);
Polylactides (PLA);
Stereo-copolymers of PLA such as
Poly-L-lactide (PLLA)
Poly-DL-lactide (PDLLA)
L-lactide/DL-lactide copolymers;
Copolymers of PLA such as
Lactide/terramethylglycolide copolymers
Lactide/trimethylene carbonate copolymers
Lactide / δ -valerolactone copolymers
Lactide/ ϵ -caprolactone copolymers;
Polydepsipeptides;
PLA/polyethylene oxide copolymers;
Unsymmetrical 3,6-substituted poly-1,4-dioxane-2,5-diones;
Poly- β -hydroxybutyrate (PHBA);
PHBA/ β -hydroxyvalerate copolymers (PHBA/HVA);
Poly-p-dioxanone (PDS);
Poly- δ -valerolactone;
Poly- ϵ -caprolactone;
Methylmethacrylate-N-vinyl pyrrolidine copolymers;
Polyesteramides;
Polyesters of oxalic acid;
Polydihydropyranes;
Polyalkyl-2-cyanoacrylates;
Polyurethanes (PU);
Polyvinylalcohol (PVA);
Polypeptides;
Poly- β -malic acid (PMLA);
Poly- β -alcanoic acids;
in combination, with the ligands to the receptor/binding proteins.

OPI DATE 12/06/90

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PCT NUMBER PCT/SE89/00666

PCT

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : A61K 9/22, 47/00, 37/02 A61K 37/36 // A61L 27/00	A1	(11) International Publication Number: WO 9 /05522 (43) International Publication Date: 31 May 1990 (31.05.90)
(21) International Application Number: PCT/SE89/00666 (24) International Filing Date: 17 November 1989 (17.11.89) (30) Priority data: 8804164-5 17 November 1988 (17.11.88) SE (71)(72) Applicants and Inventors: PRISELL, Per [SE/SE]; Wollmar Yxkullsgatan 15A, S-116 50 Stockholm (SE). NORSTEDT, Gunnar [SE/SE]; Hantverkargatan 76D, S-112 38 Stockholm (SE). (74) Agents: BERGVALL, Stina, Lena et al.; Dr. Ludwig Brann Patentbyrå AB, P.O. Box 7524, S-103 92 Stockholm (SE). (81) Designated States: AT (European patent), AU, BB, BE (Eu- ropean patent), BF (OAPI patent), BG, BJ (OAPI pa- tent), BR, CF (OAPI patent), CG (OAPI patent), CH (European patent), CM (OAPI patent), DE (European patent), DK, ES (European patent), FI, FR (European patent), GA (OAPI patent), GB (European patent), HU, IT (European patent), JP, KP, KR, LK, LU (European patent), MC, MG, ML (OAPI patent), MR (OAPI pa- tent), MW, NL (European patent), NO, RO, SD, SE (Eu- ropean patent), SN (OAPI patent), SU, TD (OAPI pa- tent), TG (OAPI patent), US.		Published <i>With international search report.</i> <i>With amended claims.</i> 632074
(54) Title: PHARMACEUTICAL PREPARATION (57) Abstract This invention relates to a pharmaceutical preparation comprising a combination of one or several receptor/binding pro- teins (binding growth factors or hormones) and hyaluronic acid (or its derivatives) or a biodegradable polymer, optionally in combination with its/these ligands (growth factors and hormones).		

PHARMACEUTICAL PREPARATION.

The present invention is relating to pharmaceutical preparations consisting of one or several carriers in combination with one or several receptors/binding proteins. The receptors/binding proteins might optionally be connected to suitable ligands, e.g. growth hormone.

By this combination; carrier+receptor/binding protein+e.g. active peptide, according to the invention, a slow release preparation is obtained. Reversely, the principles according to this invention might also be useful in the situation of abnormal, increased production of growth factors, e.g. tumour growth, this time carrier+receptor/binding protein acting as a selective resorption agent of growth factors.

Receptors are protein molecules that can bind hormones and growth factors (=ligands). Each receptor type is specific for its ligand. The receptor function is to convey external e.g. hormonal signals into the target cell. Novel achievements in receptor research have resulted in the possibility to obtain large quantities of specific pure receptor protein. This possibility is fundamental for the present invention which hereby defines a novel therapeutical role of receptors. Some of the known receptors can be exemplified by : Insulin-like growth factor-1-receptor, Insulin-like growth factor-2-receptor, Insulin-receptor, Platelet derived growth factor-receptor, Fibroblast growth factor-receptor, Epidermal growth factor-receptor, Nerve growth factor-receptor, Colony stimulating factor-receptor, Transforming growth factor-receptors, Growth hormone-receptor, Parathyroid hormone-receptor, Calcitonin-receptor, Estrogen-receptor, Tumour necrosis factor-receptor, Insulin-like growth factor serum binding protein and Corticosteroid binding globulin.

It is generally known that growth factors and hormones, both in animals and in humans stimulates important cellular processes concerning cell division, growth, maturation, differentiation. In addition healing and regenerative processes are also regulated by these factors. The growth factors/hormones comprise e.g. Insulin-like growth factor-1 and 2; IGF-1, IGF-2, Platelet derived growth factor; PDGF, Epidermal growth factor; EGF, Fibroblast growth factor; FGF, Nerve growth factor; NGF, Colony stimulating factor; CSF, Transforming growth factor; TGF, Tumour necrosis factor; TNF, Calcitonin; CT, Parathyroid hormone; PTH, Growth

hormone; GH, Estrogens, Bombesin, Bone morphogenetic protein; BMP, Insulin and Corticosteroids.

Insulin, estrogens, corticosteroids, Ct and GH are all well known pharmaceutical agents in the daily clinical practice. The research is intense concerning the other
5 different previously mentioned growth factors and hormones. Relating to various studies and results e.g. PDGF and IGF-1 potentiates wound healing, GH increases fracture healing etc. Although most of these growth factors and hormones do have interesting effects according to different recorded data, clinical implications are yet to be found. The results as hitherto obtained indicates that the ways of
10 administration, as presently used, restrict the use of the substances as drugs, e.g. due to the short half-life of peptides, the potency and the potential toxicity of the substances. By the present invention, offering slow release, the bioactivity of the peptides is prolonged by its connection to the carrier materials. The carriers, such as hyaluronic acid, may also have favourable biological effects per se and e.g. a
15 polylactide rod may except being the stabilizing osteosynthetic material also act as a slow release carrier distributing fracture healing promoting polypeptides.

The present invention thus involves (two or) three components; carriers, receptors and ligands. The knowledge of each of these components is extensive (for textbook
20 information see: Ch 12 and 14 in "The molecular biology of the cell", Alberts et al., Garland publ. inc. NY and London; 1989). Based on this information it is clear that a slow release will occur for the peptides described above, if a pharmaceutical preparation is prepared according to the invention.

25 In the pharmaceutical preparations of this invention the receptor/binding protein, growth factor or hormone, and the matrix (comprising hyaluronic acid, or its derivatives, or a biodegradable polymer as described herein) may be simply admixed, or crosslinked to give requisite slow release preparations.

30 1. Cross-linking between a matrix gel e.g. hyaluronic acid and a receptor protein can be achieved by a variety of means, e.g. via imidocarbonates, carbonates, oxiranes, aziridines and activated double bonds and halogens. Such strategies have previously



2. By definition a hormone or growth factor-receptor specifically binds its ligand by high affinity and in a reversible manner and the rate of association is much faster than the dissociation rate. These receptor characteristics, high affinity, high specificity and reversibility are thus obligatory in order to use the term receptor. The dissociation rate is usually determined by incubating the receptor with radiolabelled ligands until equilibrium is reached. Excess unlabelled ligand is added and the release of non-receptorbound radioactivity measured as a function of time reveals the dissociation rate. From the above

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stated, it should be clear that the combination of a carrier, with a receptor, with a ligand gives, as a general accepted principle, a slow release of the ligand. The present invention introduces receptors in a therapeutical context, in addition it defines carriers allowing slow release.

Below is a schematic practical outline described, revealing the principles to manufacture a pharmaceutical preparation according to this invention.

Example: the preparation of a slow release GH-complex.

- a. Isolation of the extracellular domain of the GH receptor is achieved using part of GH receptor cDNA put into an expression vector driven by the MT promotor. Purification of this truncated receptor from media of transfected cells is achieved by affinity chromatography (using hGH Sepharose®) followed by a size separating gel chromatography.
- b. Hyaluronic acid is commercially available and is linked to the purified receptor by the use of the crosslinking agent, see above. The high molecular weight complex is purified from remaining GH receptor by repeated centrifugations. Around 75% of crosslinked receptor is functionally intact.
- c. The crosslinked hyaluronic acid-GH receptor preparation is then incubated with excess levels of GH for 2 hours at 37 °C, unbound hormone is removed by centrifugation.
- d. The preparation will, when injected e.g. subcutaneously, slowly release GH in a dose dependent way, not only based upon the amount of GH, but also according to the number of GH-receptors coupled to the gel. The rate of dissociation for a given preparation is determined for each batch by testing the release of immunoreactive GH in vitro (or by testing the release of radioactive GH in animal models).

A. An experiment measuring the increased body weight subsequent to different types of GH-treatment in a group of hypophysectomised (HX) rats was performed. Three HX rats were subcutaneously injected each with a hyaluronic acid-GH-receptor-GH preparation, according to the invention, containing approx 1.2 mg receptor bound GH. Three HX rats subcutaneously injected by the same amount of GH in a water solution served as controls. The slow release treated group had a significant increased bodyweight lasting more than 4 days compared to the controls.

B. A group of rats were injected in the tail by either 6 mikrogramms IGF-1+IGF-1 binding protein+Hyaluronic acid,

or 6 mikrograms IGF-1 in a water solution. The iGF-1 as used contained trace amounts (200 000 cpm) of radiolabelled IGF-1. Three rats in each group were used. After different time points the end of the tail was amputated and radioactivity analyzed. The first group of rats were found to release IGF-1 significantly slower (more than 2 days) than the controls.

Biodegradable polymers.

Examples of biodegradable polymers are listed below:

Polyglycolide (PGA)

Copolymers of glycolide:

Glycolide/L-lactide copolymers (PGA/PLLA)

Glycolide/trimethylene carbonate copolymers (PGA/TMC)

Poly lactides (PLA)

Stereo-copolymers of PLA:

Poly-L-lactide (PLLA)

Poly-DL-lactide (PDLLA)

L-lactide/DL-lactide copolymers

Copolymers of PLA:

Lactide/tetramethylglycolide copolymers

Lactide/trimethylene carbonate copolymers

Lactide / δ -valerolactone copolymers

Lactide/ ϵ -caprolactone copolymers

Polydepsipeptides

PLA/polyethylene oxide copolymers

unsymmetrical 3,6-substituted poly-1,4-dioxane-2,5-diones

Poly- β -hydroxybutyrate (PHBA)

PHBA/ β -hydroxyvalerate copolymers (PHBA/HVA)

Poly-p-dioxanone (PDS)

Poly- δ -valerolactone

Poly- ϵ -caprolactone

Methylmethacrylate-N-vinyl pyrrolidine copolymers

Polyesteramides

Polyesters of oxalic acid

Polydihydropyranes

Polyalkyl-2-cyanoacrylates

Polyurethanes (PU)

Polyvinylalcohol (PVA)

PolypeptidesPoly- β -malic acid (PMLA)Poly- β -alcanoic acids.

Within the body all of these are degraded by hydrolysis. The different polymers varies in their structural and chemical aspects which gives them different strength, action, degradation-time and usefulness. PDS for instance is used as a resorptive suture-material. The PGA is used in osteosynthetic material as rods, plates and screws e.g. the Biofix®. PLLA ligaments are used, in research, as replacement for the anterior cruciate ligament of the knee etc.

A combination of the different principles, i.e. the stabilizing osteosynthetic material in combination with a local slow release of e.g. fracture-healing promoting peptides, is attractive. The biodegradable polymers structurally being porous and having different grade of coating facilitates the absorption of the growth factor+receptor complex.

C. In an experiment, according the principles above, PGA rods (1.5 x 8 mm) were "loaded" with radiolabelled IGF-1+IGF-1 binding protein (see p. B) using vacuum forces, end parts sealed by melting. The rods were implanted in the end of the tail in three rats. Three control rats were injected in the same part of the tail using the same amount of radiolabelled IGF-1 in a water solution. The slow release rats, according to the invention, had a significant higher radioactivity in tails compared to the controls.

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Claims:

1. Pharmaceutical preparation for use in vivo to achieve slow release of growth factors and hormones, characterized in that it is a combination of: one or several receptor/binding proteins, optionally modified, ~~such as truncated~~, for binding growth factors or hormones, selected from the group, consisting of
Insulin-like growth factor-1-receptor;
Insulin-like growth factor-2-receptor;
Insulin-receptor;
Platelet derived growth factor-receptor;
Fibroblast growth factor-receptor;
Epidermal growth factor-receptor;
Nerve growth factor-receptor;
Colony stimulating factor-receptor;
Transforming growth factor-receptors;
Growth hormone-receptor;
Parathyroid hormone-receptor;
Calcitonin-receptor;
Estrogen-receptor;
Tumour necrosis factor-receptor;
Insulin-like growth factor serum binding protein;
Corticosteroid binding globulin;
and hyaluronic acid, or its derivatives, or a biodegradable polymer, selected from the group consisting of
Polyglycolide (PGA);
Copolymers of glycolide such as
Glycolide/L-lactide copolymers (PGA/PLLA)
Glycolide/trimethylene carbonate copolymers (PGA/TMC);
Polylactides (PLA);
Stereo-copolymers of PLA such as
Poly-L-lactide (PLLA)
Poly-DL-lactide (PDLLA)
L-lactide/DL-lactide copolymers;
Copolymers of PLA such as
Lactide/tetramethylglycolide copolymers
Lactide/trimethylene carbonate copolymers
Lactide / δ -valerolactone copolymers
Lactide/ ϵ -caprolactone copolymers;
Polydepsipeptides;
PLA/polyethylene oxide copolymers;
Unsymmetrical 3,6-substituted poly-1,4-dioxane-2,5-diones;
Poly- β -hydroxybutyrate (PHBA);
PHBA/ β -hydroxyvalerate copolymers (PHBA/HVA);



SUBSTITUTE

Poly-p-dioxanone (PDS);
Poly- δ -valerolactone;
Poly- ϵ -caprolactone;
Methylmethacrylate-N-vinyl pyrrolidine copolymers;
Polyesteramides;
Polyesters of oxalic acid;
Polydihydropyranes;
Polyalkyl-2-cyanoacrylates;
Polyurethanes (PU);
Polyvinylalcohol (PVA);
Polypeptides;
Poly- β -malic acid (PMLA);
Poly- β -alcanoic acids;
in combination, with the ligands to the receptor/binding proteins.

2. Pharmaceutical preparation, according to claim 1, characterized in that the receptors/binding proteins are mixed with or covalently bound to the hyaluronic acid or the biodegradable polymer.

3. Pharmaceutical preparation, according to claim 1, characterized in that the ligands e.g. are:
Insulin-like growth factor-1 and 2 (IGF-1, IGF-2);
Platelet derived growth factor (PDGF);
Epidermal growth factor (EGF);
Fibroblast growth factor (FGF);
Nerve growth factor (NGF);
Colony stimulating factor (CSF);
Transforming growth factor (TGF);
Tumour necrosis factor (TNF);
Calcitonin (CT);
Parathyroid hormone (PTH);
Growth hormone (GH);
Estrogens;
Bombesin;
Bone morphogenetic protein (BMP);
Corticosteroids;
Insulin.



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4. Pharmaceutical preparation according to any one of claims 1 to 3 characterized in that the receptor/binding proteins are truncated.
5. DATED this 19th day of October, 1992.

PER PRISELL and GUNNAR NORSTEDT

By Their Patent Attorneys

DAVIES COLLISON CAVE



INTERNATIONAL SEARCH REPORT

International Application No PCT/SE 89/00666

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 IPC5: A 61 K 9/22, 47/00, 37/02, 37/36//A 61 L 27/00		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁷		
Classification System ¹	Classification Symbols	
IPC5	A 61 K; A 61 L; C 07 K	
Documentation Searched other than Minimum Documentation to the extent that such Documents are included in the Fields Searched ⁸		
SE,DK,FI,NO classes as above		
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
P,X	WO, A1, 88/09818 (GENENTECH INC.) 15 December 1988, see page 17 lines 26-35; example 8; claim 31 --	1-3,5
X	US, A, 4687808 (R. D. JARRETT ET AL.) 18 August 1987, see claims 1-5; column 5 lines 25-61 --	1,2,4
X	US, A, 4469681 (M. BROWNLEE ET AL.) 4 September 1984, see column 5 line 10 - column 6 line 2; column 6 line 61 - column 7 line 10; column 8 lines 7-43 --	1,2
¹⁰ Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. "A" document member of the same patent family		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
24th January 1990	1990 -01- 2 9	
International Searching Authority	Signature of Authorized Officer	
SWEDISH PATENT OFFICE	Niklas Forslund	

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
P,X	WO, A1, 89/04838 (IMMUNEX CORPORATION) 1 June 1989, see especially claim 23 --	1,2
P,X	US, A, 4828563 (W.G.K. MÜLLER-LIERHEIM) 9 May 1989, see the whole document --	1-5
X	EP, A2, 0103184 (DIAMOND SHAMROCK CORPORATION) 21 March 1984, see page 9 and claims --	1,2,4
X	EP, A2, 0014995 (PHARMACIA AB) 3 September 1980, see the whole document --	1
X	WO, A1, 88/07078 (DR. MÜLLER-LIERHEIM AG) 22 September 1988, see the whole document -----	1,2,4

**ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO. PCT/SE 89/00666**

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A1- 88/09818	15/12/88	AU-D- 19865/88	04/01/89
US-A- 4687803	18/08/87	EP-A- 0103184	21/03/84
		AU-D- 17965/83	23/02/84
		JP-A- 59049773	22/03/84
		CA-A- 1217424	03/02/87
		AU-A- 570213	10/03/83
		US-A- 4737544	12/04/88
US-A- 4469681	04/09/84	WO-A- 81/00354	19/02/81
		EP-A-B- 0032927	05/08/81
		US-A- 4348387	07/09/82
		AU-D- 61290/80	03/03/81
		CA-A- 1168983	12/06/84
		AT-E- 10165	15/11/84
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WO-A1- 89/04838	01/06/89	EP-A- 0318296	31/05/89
		AU-D- 29015/89	14/06/89
US-A- 4828563	09/05/89	EP-A- 0205790	30/12/86
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		JP-A- 62049856	04/03/87
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		US-A- 4789634	06/12/88
EP-A2- 0103184	21/03/84	AU-D- 17965/83	23/02/84
		JP-A- 59049773	22/03/84
		CA-A- 1217424	03/02/87
		US-A- 4687808	18/08/87
		AU-A- 570213	10/03/88
		US-A- 4737544	12/04/88
EP-A2- 0014995	03/09/80	JP-A- 55120520	17/09/80
		SE-A- 7901565	22/08/80
WO-A1- 88/07078	22/09/88	DE-A- 3709101	29/09/88