PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 38/36, 38/49	A1	(11) International Publication Number: **WO 95/12407** (43) International Publication Date: 11 May 1995 (11.05.95)

(21) International Application Number: PCT/DK94/00414

(22) International Filing Date: 3 November 1994 (03.11.94)

(30) Priority Data:
1256/93 5 November 1993 (05.11.93) DK

(71) Applicant *(for all designated States except US)*: NOVO NORDISK A/S [DK/DK]; Novo Allé, DK-2880 Bagsvaerd (DK).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: BREGENGAARD, Claus [DK/DK]; Højsgaards Allé 72, DK-2900 Hellerup (DK).

(74) Agent: THALSØ-MADSEN, Birgit; Novo Nordisk A/S, Corporate Patents, Novo Allé, DK-2880 Bagsvaerd (DK).

(81) Designated States: AU, CA, CN, CZ, FI, HU, JP, KR, NO, PL, RU, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: A PHARMACEUTICAL COMPOSITION FOR THE TREATMENT OF CORONARY THROMBOSIS

(57) Abstract

A method of treating coronary thrombosis, the method comprising administering, to a patient in need of such treatment, an effective amount of lys-plasminogen and, simultaneously or sequentially, an effective amount of tissue plasminogen activator.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	**GB**	United Kingdom	**MR**	Mauritania
AU	Australia	**GE**	Georgia	**MW**	Malawi
BB	Barbados	**GN**	Guinea	**NE**	Niger
BE	Belgium	**GR**	Greece	**NL**	Netherlands
BF	Burkina Faso	**HU**	Hungary	**NO**	Norway
BG	Bulgaria	**IE**	Ireland	**NZ**	New Zealand
BJ	Benin	**IT**	Italy	**PL**	Poland
BR	Brazil	**JP**	Japan	**PT**	Portugal
BY	Belarus	**KE**	Kenya	**RO**	Romania
CA	Canada	**KG**	Kyrgystan	**RU**	Russian Federation
CF	Central African Republic	**KP**	Democratic People's Republic of Korea	**SD**	Sudan
CG	Congo			**SE**	Sweden
CH	Switzerland	**KR**	Republic of Korea	**SI**	Slovenia
CI	Côte d'Ivoire	**KZ**	Kazakhstan	**SK**	Slovakia
CM	Cameroon	**LI**	Liechtenstein	**SN**	Senegal
CN	China	**LK**	Sri Lanka	**TD**	Chad
CS	Czechoslovakia	**LU**	Luxembourg	**TG**	Togo
CZ	Czech Republic	**LV**	Latvia	**TJ**	Tajikistan
DE	Germany	**MC**	Monaco	**TT**	Trinidad and Tobago
DK	Denmark	**MD**	Republic of Moldova	**UA**	Ukraine
ES	Spain	**MG**	Madagascar	**US**	United States of America
FI	Finland	**ML**	Mali	**UZ**	Uzbekistan
FR	France	**MN**	Mongolia	**VN**	Viet Nam
GA	Gabon				

A pharmaceutical composition for the treatment of coronary thrombosis

FIELD OF INVENTION

The present invention relates to a method of treating coronary thrombosis, a method of preventing reocclusion of coronary arteries, as well as a pharmaceutical composition for use in the methods.

BACKGROUND OF THE INVENTION

The use of plasminogen activators such as urokinase, streptokinase and tissue plasminogen activator as the sole fibrinolytic agents in the treatment of thrombosis is well known, for instance as reviewed in E.J. Topol, "Advances in Thrombolytic Therapy for Acute Myocardial Infarction", J. Clin. Pharmacol. 27, 1987, pp. 735-745; V.J. Marder and S. Sherry, "Thrombolytic Therapy: Current Status", New England J. Med. 318, 1988, pp. 1585-1595; J. Loscalzo, "Thrombolysis in the Management of Acute Myocardial Infarction and Unstable Angina Pectoris", Drugs 37, 1989, pp. 191-204; and S. Sherry, J. Int. Med. 229, 1991, pp. 113-116. The use of lys-plasminogen as a thrombolytic agent has been proposed previously by K. Anderle et al., Haemostasis 18, 1988, pp. 165-175, reviewing studies of the systemic use for the treatment of deep venous thrombosis of lys-plasminogen activated by urokinase and streptokinase. Lys-plasminogen in combination with urokinase is also proposed for the treatment of pulmonary embolism.

S.F. Badylak et al., Haemostasis 21, 1991, pp. 278-285, and S.F. Badylak et al., Thrombosis Research 62, 1991, pp. 115-126 report the enhancement of the thrombolytic effect of urokinase by lys-plasminogen in a dog model of arterial thrombosis.

SUMMARY OF THE INVENTION

The combination of lys-plasminogen with tissue plasminogen activator for the treatment of coronary thrombosis is believed to be novel.

Accordingly, the present invention relates to a method of treating coronary thrombosis, the method comprising administering, to a patient in need of such treatment, an effective amount of lys-plasminogen and, simultaneously or sequentially, an effective amount of tissue plaminogen activator (t-PA).

It was surprisingly found (*vide* the study described below in Example 1) that when lys-plasminogen was administered in combination with t-PA, the combined treatment not only reduced the time to restoration of flow, but also prevented reocclusion of the coronary artery. Reocclusion of coronary arteries represents a major problem in thrombolytic therapy. Although early and complete restoration of coronary patency may result in substantial salvage of myocardium during infarction, this initial benefit may be attenuated by subsequent reocclusion. Reocclusion rates vary from 10 to 15% (cf. D. Collem et al, Drugs 38, 1989, 346-388)., and patients with reoccluded arteries frequently suffer from reinfarction (cf. S.H. Schaer et al., Circulation 76 (Suppl. II), 1987, pp. 57-62. Also, the time to reperfusion seems to be highly important for the outcome of thrombolytic therapy. Thus, accelerated t-PA treatment resulting in a more rapid infarction-artery patency during thrombolytic therapy is associated with a lower mortality rate (cf. The GUSTO investigators. An Internal Randomized Trial Comparing four Thrombolytic Strategies for Acute Myocardial Infarction, New England J. Med. 329, 1993, pp. 673-682). Thus, the finding that the combined administration of lys-plasminogen and t-PA is effective to decrease the time to reperfusion and prevent reocclusion of the coronary artery is of vital importance for the efficient and lasting treatment of coronary thrombosis.

Theoretically, accelarated thrombolysis and prevention of reocclusion may furthermore result in decreased secondary restenosis in the vessels due to shorter exposure to the vessel wall to inducers of cell proliferation known to be produced by the thrombi (for instance PDGF, thrombin, etc.).

In another aspect, the present invention therefore relates to a method of preventing reocclusion of coronary arteries following treatment of coronary thrombosis, the method comprising administering, to a patient in need of such treatment, an effective amount

of lys-plasminogen and, simultaneously or sequentially, an effective amount of tissue plaminogen activator (t-PA).

In a further aspect, the invention relates to a pharmaceutical composition for the treatment of coronary thrombosis, the composition comprising, in separate containers, lys-plasminogen and t-PA, each together with a pharmaceutically acceptable carrier or diluent.

In a still further aspect, the invention relates to the use of lys-plasminogen in combination with t-PA for the preparation of a medicament for the treatment of coronary thrombosis as well as for the prevention of reocclusion of coronary arteries following such treatment.

DETAILED DESCRIPTION OF THE INVENTION

Lys-plasminogen for use according to the present invention may be prepared from native plasminogen (glu-plasminogen) by brief exposure of glu-plasminogen to plasmin resulting in cleavage of 77 amino-terminal amino acids and the formation of lys-plasminogen (named for the N-terminal lysine).

Native human plasminogen may be purified from plasma by several methods. Recent methods of purification from human plasma are based on affinity chromatography as described by D.G. Deutsch and E.T.Mertz, Fed. Proc. 29, 1979, p. 647, and Science 170, 1970, pp. 1095-1096. An affinity matrix (lysine-Sepharose®) is prepared by covalent coupling of the α-amino group of L-lysine to Sepharose®. Plasma diluted with water is passed through a lysine-Sepharose® column equilibrated with phosphate buffer (pH7.4) at room temperature after which the column is washed with phosphate buffer. Plasminogen is then eluted with 0.2 M ϵ-amino caproic acid (pH 7.4). The ϵ-amino caproic acid is then removed from plasminogen in the cold, by gel filtration on Sephadex® equilibrated with phosphate buffer.

The use of human proteins purified from human plasma involves a certain risk of viral infection. In particular, the risk of HIV and hepatitis infections has become a major concern in dealing with plasma-derived human proteins. To avoid such risk, it is therefore preferable to produce plasminogen by recombinant DNA techiques. cDNA sequences encoding plasminogen and lys-plasminogen as well as the preparation thereof are described in EP 319 944 (Zymogenetics).

Likewise, it is preferred to produce the t-PA to be used according to the present invention by recombinant DNA techniques. cDNA encoding t-PA has been described by Pennica et al., Nature 301, 1983, pp. 214-221; Kaufman et al., Mol. Cell. Biol. 5, 1985, pp. 1750-1759; US 4,766,075; and Verheijen et al, EMBO J. 5, 1986, pp. 3525-3530. In stead of native t-PA, a t-PA analogue may be used according to the invention. Examples of t-PA analogues are described in, e.g. D.L. Higgins and W.F. Bennett, Ann. Rev. Pharmacol. Toxicol. 30, 1990, pp. 91-121; L. Pierard and A. Bollen, J. Biotechnology 15, 1990, pp. 283-304; and H. Pannekoek et al., Fibrinolysis 2, 1988, pp. 123-132.

The DNA coding for the desired polypeptide (*in casu* plasminogen, lys-plasminogen, t-PA or t-PA analogue) may be introduced into a suitable recombinant expression vector. This may be any vector which may conveniently be subjected to recombinant DNA procedures, and the choice of vector will often depend on the host cell into which it is to be introduced. Thus, the vector may be an autonomously replicating vector, i.e. a vector which exists as an extrachromosomal entity, the replication of which is independent of chromosomal replication, e.g. a plasmid. Alternatively, the vector may be one which, when introduced into a host cell, is integrated into the host cell genome and replicated together with the chromosome(s) into which it has been integrated.

In the vector, the DNA sequence encoding the desired polypeptide should be operably connected to a suitable promoter sequence. The promoter may be any DNA sequence which shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. Examples of suitable promoters for directing the transcription of the DNA encoding the desired

polypeptide in mammalian cells are the SV40 promoter (Subramani et al., Mol. Cell Biol. 1 (1981), 854 -864), the MT-1 (metallothionein gene) promoter (Palmiter et al., Science 222 (1983), 809 - 814) or the adenovirus 2 major late promoter. A suitable promoter for use in insect cells is the polyhedrin promoter (Vasuvedan et al., FEBS Lett. 311, (1992) 7 - 11). Suitable promoters for use in yeast host cells include promoters from yeast glycolytic genes (Hitzeman et al., J. Biol. Chem. 255 (1980), 12073 - 12080; Alber and Kawasaki, J. Mol. Appl. Gen. 1 (1982), 419 - 434) or alcohol dehydrogenase genes (Young et al., in Genetic Engineering of Microorganisms for Chemicals (Hollaender et al, eds.), Plenum Press, New York, 1982), or the TPI1 (US 4,599,311) or ADH2-4c (Russell et al., Nature 304 (1983), 652 - 654) promoters. Suitable promoters for use in filamentous fungus host cells are, for instance, the ADH3 promoter (McKnight et al., The EMBO J. 4 (1985), 2093 - 2099) or the tpiA promoter.

The DNA sequence encoding the desired polypeptide may also be operably connected to a suitable terminator, such as the human growth hormone terminator (Palmiter et al., op. cit.) or (for fungal hosts) the TPI1 (Alber and Kawasaki, op. cit.) or ADH3 (McKnight et al., op. cit.) terminators. The vector may further comprise elements such as polyadenylation signals (e.g. from SV40 or the adenovirus 5 Elb region), transcriptional enhancer sequences (e.g. the SV40 enhancer) and translational enhancer sequences (e.g. the ones encoding adenovirus VA RNAs).

The recombinant expression vector may further comprise a DNA sequence enabling the vector to replicate in the host cell in question. An example of such a sequence (when the host cell is a mammalian cell) is the SV40 origin of replication. The vector may also comprise a selectable marker, e.g. a gene the product of which complements a defect in the host cell, such as the gene coding for dihydrofolate reductase (DHFR) or one which confers resistance to a drug, e.g. neomycin, hygromycin or methotrexate.

The procedures used to ligate the DNA sequences coding for the desired polypeptide, the promoter and the terminator, respectively, and to insert them into suitable vectors containing the information necessary for replication, are well known to persons skilled

in the art (cf., for instance, Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor, New York, 1989).

The host cell into which the expression vector is introduced may be any cell which is capable of producing the desired polypeptide and may suitably be a eukaryotic cell, such as invertebrate (insect) cells or vertebrate cells, e.g. Xenopus laevis oocytes or mammalian cells, in particular insect and mammalian cells. Examples of suitable mammalian cell lines are the COS (ATCC CRL 1650), BHK (ATCC CRL 1632, ATCC CCL 10), CHL (ATCC CCL39) or CHO (ATCC CCL 61) cell lines. Methods of transfecting mammalian cells and expressing DNA sequences introduced in the cells are described in e.g. Kaufman and Sharp, J. Mol. Biol. 159 (1982), 601 - 621; Southern and Berg, J. Mol. Appl. Genet. 1 (1982), 327 - 341; Loyter et al., Proc. Natl. Acad. Sci. USA 79 (1982), 422 - 426; Wigler et al., Cell 14 (1978), 725; Corsaro and Pearson, Somatic Cell Genetics 7 (1981), 603, Graham and van der Eb, Virology 52 (1973), 456; and Neumann et al., EMBO J. 1 (1982), 841 - 845.

Alternatively, fungal cells (including yeast cells) may be used as host cells of the invention. Examples of suitable yeasts cells include cells of Saccharomyces spp. or Schizosaccharomyces spp., in particular strains of Saccharomyces cerevisiae. Examples of other fungal cells are cells of filamentous fungi, e.g. Aspergillus spp. or Neurospora spp., in particular strains of Aspergillus oryzae or Aspergillus niger. The use of Aspergillus spp. for the expression of proteins is described in, e.g., EP 238 023.

The desired polypeptide may then be produced by a method which comprises culturing a cell as described above in a suitable nutrient medium under conditions which are conducive to the expression of the desired polypeptide and recovering the resulting polypeptide from the culture.

The medium used to culture the cells may be any conventional medium suitable for growing mammalian cells, such as a serum-containing or serum-free medium containing appropriate supplements. Suitable media are available from commercial suppliers or

may be prepared according to published recipes (e.g. in catalogues of the American Type Culture Collection).

Attempts to produce plasminogen in transfected mammalian cells have, however, shown that intracellular plasminogen activation and subsequent degradation provides an obstacle to the production of intact recombinant plasminogen in reasonable yields. The problem has been solved by coexpression of plasminogen with a protease inhibitor capable of inhibiting plasminogen activation, substantially as described in EP 319 944.

Thus, lys-plasminogen or glu-plasminogen may be produced by coexpression of lys- or glu-plasminogen with α-2-plasmin inhibitor or α-1-antitrypsin (AAT) in transfected BHK cells. Coexpression with AAT causes inhibition of urokinase produced by the BHK cells. Activation of plasminogen by urokinase and plasmin-mediated proteolytic cleavage is thereby greatly reduced and consequently, high levels of intact plasminogen is obtained.

The polypeptide produced by the cells may then be recovered from the culture medium by conventional procedures including separating the host cells from the medium by centrifugation or filtration, precipitating the proteinaceous components of the supernatant or filtrate by means of a salt, e.g. ammonium sulphate, purification by a variety of chromatographic procedures, e.g. ion exchange chromatography, affinity chromatography, or the like.

The pharmaceutical composition of the invention may be compounded in any form suitable for parenteral administration (i.e. for intravenous injection or infusion), for instance by dissolving or suspending lys-plasminogen and t-PA, respectively, in sterile water or isotonic saline. In order to obtain the desired thrombolytic effect of the composition, it is contemplated that the suitable dosage of lys-plasminogen may be in the range of 0.1-3.0 mg/kg body weight, preferably 0.3-1.5 mg/kg body weight, more preferably about 1 mg/kg body weight, dependent on the severity of the condition, the age and general health of the patient to whom the composition is administered, etc. A corresponding suitable dosage of t-PA is one which is capable of activating the lys-

plasminogen administered to the patient. Thus, the dosage of t-PA co-administered with lys-plasminogen may suitably be in the range of 0.2-2.0 mg/kg body weight, preferably 0.3-1.5 mg/kg body weight, more preferably about 1 mg/kg body weight. This means that the unit dosage of lys-plasminogen in the composition will typically be in the range of 20-100 mg, and the unit dosage of t-PA will typically be in the range of 20-100 mg.

In one embodiment of the method of the invention, the administration of lys-plasminogen may be substantially simultaneous with the administration of t-PA. This may, for instance, be effected by providing lys-plasminogen and t-PA in a device which makes it possible to administer two substances simultaneously. Alternatively, either lys-plasminogen or t-PA may be administered first and the other component may be administered immediately after that ("immediately" meaning any period of time up to one minute after administration of the first component).

In another embodiment, t-PA may be administered before the administration of lys-plasminogen.

In a further, currently preferred embodiment, lys-plasminogen may be administered before the administration of t-PA. To obtain the desired activation of lys-plasminogen by t-PA, it is contemplated that the t-PA may be adminstered 5-20 minutes, preferably about 10 minutes, after the administration of lys-plasminogen.

In a still further aspect, the present invention relates to the use of lys-plasminogen in combination with t-PA for the preparation of a medicament for the treatment of coronary thrombosis. For this purpose, the lys-plasminogen and t-PA are provided in separate containers in a form adapted to the simultaneous or sequential co-administration of lys-plasminogen and t-PA.

The invention is further illustrated in the following examples which are not in any way intended to limit the scope of the invention as claimed.

Example 1

In vivo study of the thrombolytic effect of lys-plasminogen on t-PA-induced thrombolysis

Recombinant human lys-plasminogen was expressed in BHK cells substantially as described in EP 319 944 and purified by the method described in PCT/DK93/00206. The purified product was freeze-dried by conventional methods. The freeze-dried lys-plasminogen was stored in vials containing 50 mg protein per vial.

T-PA was obtained from Boehringer Ingelheim.

15 dogs with a mean body weight of 20 kg were included in the study. Formation of coronary thrombi was induced electrically in the LAD coronary artery. After the thrombi had been stable for 20 minutes, the dogs were treated with either saline (n=10) or 2 mg/kg lys-plasminogen (n=5). Lys-plasminogen or saline did not induce reflow in any of the dogs. Ten minutes later, t-PA (1 mg/kg over 20 minutes) was infused and blood flow characteristics were observed over 2 hours.

Time to restoration of flow was less in the lys-plasminogen-treated dogs (14 +/-4 vs. 58 +/-19 minutes in saline-treated dogs, mean +/- SD $P<0.001$), and the peak restored flow was greater in the lys-plasminogen-treated dogs (73 +/-22 vs. 47 +/-28 ml/minute). The restored flow persisted in all lys-plasminogen-treated dogs and in only 2 out of 10 saline-treated dogs over the two-hour period (reocclusion rates 0 vs. 80%, $P<0.001$). Myocardial shortening fraction at 2 hours after t-PA infusion in the LAD supplied region in lys-plasminogen-treated dogs was +1 +/-1% (vs. -10 +/-5% in saline-treated dogs, $P<0.01$). Plasma fibrinogen levels were lower in lys-plasminogen-treated dogs (0.3 +/-0.1 vs. 1.5 +/-0.0 g/l, $P<0.01$) at 2 hours after t-PA infusion. The α-2-antiplasmin levels were also lower (29 +/-5 vs. 44 +/-6% of the control).

To conclude, lys-plasminogen administered before t-PA reduces the time to restoration of flow, sustains the restored flow and preserves myocardial function.

CLAIMS

1. A method of treating coronary thrombosis, the method comprising administering, to a patient in need of such treatment, an effective amount of lys-plasminogen and, simultaneously or sequentially, an effective amount of tissue plaminogen activator (t-PA).

2. A method according to claim 1, wherein the effective amount of lys-plasminogen is in the range of 0.1-3.0 mg/kg body weight, preferably 0.3-1.5 mg/kg body weight, more preferably about 1 mg/kg body weight.

3. A method according to claim 1 or 2, wherein the effective amount of t-PA is in the range of 0.2-2.0 mg/kg body weight, preferably 0.3-1.5 mg/kg body weight, more preferably about 1 mg/kg body weight.

4. A method according to claim 1, wherein the administration of lys-plasminogen is followed, after a suitable interval, by administration of t-PA.

5. A method according to claim 4, wherein said suitable interval is in the range of 0-120 minutes, preferably about 5 minutes.

6. A pharmaceutical composition for the treatment of coronary thrombosis, the composition comprising, in separate containers, lys-plasminogen and t-PA, each together with a pharmaceutically acceptable carrier or diluent.

7. A pharmaceutical composition according to claim 6, which contains 20-100 mg of lys-plasminogen.

8. A pharmaceutical composition according to claim 6, which contains 20-100 mg t-PA.

9. Use of lys-plasminogen in combination with t-PA for the preparation of a medicament for the treatment of coronary thrombosis.

10. Use according to claim 9, wherein the lys-plasminogen and t-PA are provided in separate containers in a form adapted to the simultaneous or sequential co-administration of lys-plasminogen and t-PA.

11. Use according to claim 10, wherein the lys-plasminogen is present in an amount of 20-100 mg, and wherein the t-PA is present in an amount of 20-100 mg.

12. A method of preventing reocclusion of coronary arteries following treatment of coronary thrombosis, the method comprising administering, to a patient in need of such treatment, an effective amount of lys-plasminogen and, simultaneously or sequentially, an effective amount of tissue plaminogen activator (t-PA).

13. A method according to claim 12, wherein the effective amount of lys-plasminogen is in the range of 0.1-3.0 mg/kg body weight, preferably 0.3-1.5 mg/kg body weight, more preferably about 1 mg/kg body weight.

14. A method according to claim 12 or 13, wherein the effective amount of t-PA is in the range of 0.2-2.0 mg/kg body weight, preferably 0.3-1.5 mg/kg body weight, more preferably about 1 mg/kg body weight.

15. A method according to claim 12, wherein the administration of lys-plasminogen is followed, after a suitable interval, by administration of t-PA.

16. A method according to claim 15, wherein said suitable interval is in the range of 0-120 minutes, preferably about 5 minutes.

17. Use of lys-plasminogen in combination with t-PA for the preparation of a medicament for the prevention of reocclusion of coronary arteries following treatment of coronary thrombosis.

18. Use according to claim 17, wherein the lys-plasminogen and t-PA are provided in separate containers in a form adapted to the simultaneous or sequential co-administration of lys-plasminogen and t-PA.

19. Use according to claim 18, wherein the lys-plasminogen is present in an amount of 20-100 mg, and wherein the t-PA is present in an amount of 20-100 mg.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 94/00414

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: A61K 38/36, A61K 38/49
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

MEDLINE, BIOSIS, EMBASE, WPIL, WPI, US PATENTS FULLTEXT DATABASES, DIALINDEX

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CIRCULATION, Volume 88, No 4, October 1993, J. L. Metha et al, "Lys-plasminogen Improves the Quality of Tissue-Plasminogen Activator-Induced Coronary Thrombolysis in Dogs" page 616 --	6-11,17-19
X	Dialog Information Services, file 155, Medline, Dialog Accession no. 07079611, Medline accession no. 89381611, Marcon JL: "Local thrombolysis using a combination of RTPA and Lys-plasminogen in ischemia due to a previous thrombosisa", & J Mal Vasc 1989, 14 (3) p265-6 --	6-11,17-19

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts 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 when the document is taken alone
"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
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 February 1995	22 -02- 1995
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Carolina Palmcrantz Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 94/00414

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Dialog Information Services, file 155, Medline, Dialog Accession no. 06981990, Medline accession no. 89283990, Ljungberg J et al: "Potentiated thrombolysis in a Chandler system using rtPA and lys-plasminogen", Thromb Res Mar 15 1989, 53 (6) p569-76	6-11,17-19
X	Dialog Information Services, file 155, Medline, Dialog accession no. 08041983, Medline accession no. 92179983, Nishino N et al: "Influence of intrinsic and extrinsic plasminogen upon the lysis of thrombi in vitro", & Thromb Haemost Dec 2 1991, 66 (6) p672-7	6-11,17-19
X	Dialog Information Services, file 155, Medline, Dialog accession no. 07374315, Medline accession no. 90281315, Gao SW et al: "Differential effect of platelets on plasminogen activation by tissue plasminogen activator, urokinase, and streptokinase", & Thromb Res May 15 1990, 58 (4) p421-33	6-11,17-19
P,X	Dialog Information Services, file 5, BIOSIS, Dialog accession no. 11014906, Biosis no. 97214906, Chen L et al: "Lys-plasminogen, but not Glu-plasminogen, improves the quality of tissue-plasminogen activator-induced coronary thrombolysis in dogs", & College of Cardiology, March 13-17, 1994	6-11,17-19
P,X	Dialog Information Services, file 155, Medline, Dialog accessionno. 09063195, Medline accession no. 94378195, Chen Ly et al: "Lys- and glu-plasminogen potentiate the inhibitory effect of recombinant tissue plasminogen activator on human platelet aggregation", & Thromb Res Jun 15 1994, 74 (6) p555-63	6-11,17-19

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 94/00414

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO, A1, 9115235 (SURVIVAL TECHNOLOGY, INC.), 17 October 1991 (17.10.91), page 18, line 14 - line 18 --	6-11,17-19
A	EP, A1, 0352897 (THE WELLCOME FOUNDATION LIMITED), 31 January 1990 (31.01.90), see the claims --	6-11,17-19

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 94/00414

Box I **Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 1-5, 12-16
because they relate to subject matter not required to be searched by this Authority, namely:

See PCT Rule 39.1(iv): Methods for treatment of the human or animal body by therapy.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II **Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

31/12/94

International application No.
PCT/DK 94/00414

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A1- 9115235	17/10/91	NONE	
EP-A1- 0352897	31/01/90	SE-T3- 0352897	
		AU-B- 612267	04/07/91
		AU-A- 3658489	11/01/90
		DE-U- 6890111	07/05/92
		JP-A- 2072124	12/03/90
		JP-B- 6018782	16/03/94
		US-A- 5342616	30/08/94