0 1 JUN 2005

Peptides and/or proteins as well as their use for preparing a therapeutic and/or preventive pharmaceutical composition

The invention concerns peptides and/or proteins, their use for preparing a therapeutic and/or preventive pharmaceutical composition as well as a pharmaceutical composition.

Substances for the inhibition or prevention of inflammatory reactions, so-called immunosuppressants, which so far have been used for prophylaxis and therapy, generally comprise two distinct groups. Firstly, derivatives of a hormone, i.e. cortisone, naturally occurring in the body, and secondly, exogenous immunosuppressants such as cyclosporin and its derivatives, azathioprine, cyclophosphamide etc.. All those substances possess anti-inflammatory effects but they show substantial side reactions in long-term therapy. Those side reactions have a limiting effect on long-term therapy, which is why those substances are used alternately or in combination in order to keep side effects on a tolerable level or in order to be able to actually proceed with the therapy. As examples of side reactions, the pathological fractures associated with cortison are to be mentioned, which fractures are caused by the osteoporotic effect of the cortisone, or the renal failure which may be caused by cyclosporin. Those side reactions are inevitable with both groups of compounds, and hence it is merely a question of the duration of the therapy and of the total dose at what point the therapy must be stopped.

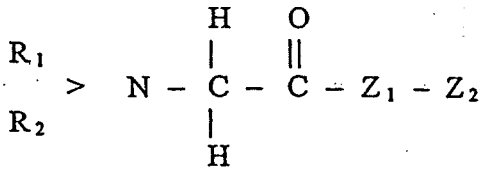
The present invention has as its object to provide new pharmaceutical products which are suitable for preventing or inhibiting inflammatory effects and which only show minor side effects. A further object consists in providing long-term therapy.

In the following, the amino acids of the peptides according to the invention are referred to by the usual abbreviations, which denote the α-amino acids.

By „analogues", a peptide is understood which, by derivatisation, substitution, preferably homologous substitution, deletion and/or insertion, is derived from the sequence of the fibrin and in particular from the preferred sequences.

The peptides or proteins according to the invention exhibit the general formula I (SEQ ID NO 1, 2)

0 1 JUN 2005



wherein R_1 and R_2, being equal or different, denote hydrogen, a saturated or unsaturated hydrocarbon residue comprising from 1 to 3, in particular up to 10, carbon atoms,

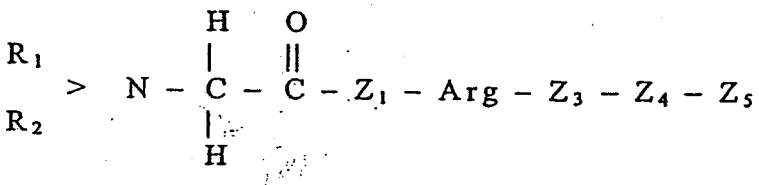
Z_1 denotes a histidine or proline residue,

Z_2 denotes an arginine residue, a peptide residue or a protein residue comprising an initial arginine residue, in particular comprising from 2 to 30 amino acids,

as well as the salts thereof, and, f.i., also amides, or mixtures with each other and/or with at least one further substance for therapeutic and/or preventive use in human and/or veterinary medicine, whereby in particular only L-amino acids are provided.

It was completely surprising that the specified amino acid sequence prevents the adhesion of cells from the bloodstream to endothelial cells of the vascular wall and/or their subsequent transmigration from the blood into the tissue.

The peptides or proteins according to the invention exhibit the general formula II (SEQ ID NO 3 to 10)



wherein R_1 and R_2, being equal or different, denote hydrogen, a saturated or unsaturated hydrocarbon residue comprising from 1 to 3, in particular up to 10, carbon atoms,

Z_1 denotes a histidine or proline residue,

Arg denotes an arginine residue

Z_3 denotes a proline or valine residue,

Z_4 denotes a leucine or valine residue,

Z_5 denotes a protein residue or a peptide residue, in particular comprising from 2 to 30 amino acids,

or an alcohol residue comprising from 1 to 3, in particular up to 10, carbon atoms,

or an organic or inorganic base residue,

01 JUN 2005

as well as the salts thereof, and, f.i., also amides, or mixtures with each other and/or with at least one further substance for therapeutic and/or preventive use in human and/or veterinary medicine, whereby in particular only L-amino acids are provided.

It was completely surprising that parts of the sequence, peptides or fragments of the fibrinogen exhibit anti-inflammatory effects. Without being bound by such theoretical considerations, said effects might be based on the fact that the fibrin binds to endothelial cells via its neo-N-terminus of the Bbeta-chain and to cells in the bloodstream via the sequence of the Aalpha-chain, thereby leading to the adhesion and transmigration of cells into the tissue. Those bindings exhibit a side reaction in that the formation of fibrin is inhibited. However, said inhibition does not constitute a potential disadvantage to the patient since the blood coagulation is sufficient also in the absence of fibrin if slight injuries occur. Only in case of surgical treatment, it might optionally be suitable to stop such kind of therapy. Other side reactions may substantially be ruled out, since those substances only interact with natural ligands. Furthermore, the natural defence is not affected adversely by the leukocytes in the blood. Thus, the composition of the same, such as granulocytes, lymphocytes and monocytes, remains unaffected so that the natural defence process is maintained and the defence against infections in the blood remains unchanged.

Fibrinogen is produced in the liver and, in this form, is biologically inactive and normally is provided in the blood at concentrations of around 3 g/l. By proteolytic cleavage of the proenzyme prothrombin, thrombin is formed which cleaves off the fibrinopeptides A and B from the fibrinogen. In doing so, fibrinogen is transformed into its biologically active form. Fibrin and fibrin cleavage products are generated.

Thrombin is formed during each activation of the blood coagulation, i.e. with each damage to the tissue, be it of inflammatory, traumatic or degenerative genesis. The formation of fibrin as mediated by thrombin is basically a protective process with the purpose of quickly sealing any defects caused to the vascular system. However, the formation of fibrin is also a pathogenic process. The appearance of a fibrin thrombus as the triggering cause of cardiac infarction is one of the most prominent problems in human medicine.

The role which fibrin plays during the extravastation of inflammatory cells from the bloodstream into the tissue, which, on the one hand, is a desired process of the defence against pathogenic microorganisms or tumour cells occurring in the tissue, but, on the other hand, is a process which, by itself, induces or prolongues damage done to the tissue, has so

0 1 JUN 2005

far not been examined at all or not to a sufficient extent. Fibrin binds to endothelial cells via its neo-N-terminus of Bbeta by means of the sequence to Bbeta and to cells in the bloodstream by means of the sequence Aalpha, thereby leading to the adhesion and transmigration of cells into the tissue.

The peptides or proteins according to the invention may prevent the adhesion of cells from the bloodstream to endothelial cells of the vascular wall and/or their subsequent transmigration from the blood into the tissue.

A peptide or protein according to the invention of the general formula II, wherein Z_5 denotes a peptide residue comprising the following amino acid sequence (SEQ ID NO 11):

Asp Lys Lys Arg Glu Glu Ala Pro Ser Leu Arg Pro Ala
Pro Pro Pro Ile Ser Gly Gly Gly Tyr Arg

and Z_1 denotes a histidine residue,
Arg denotes an arginine residue,
Z_3 denotes a proline residue,
Z_4 denotes a leucine residue,

prevents fibrin fragments from depositing on or adhering to the vascular wall. Thus, it is rendered impossible that inflammatory cells are retained at the endothelial cells of the vascular walls of arteries and veins, and such cells are prevented from remaining at the vascular walls, thus being prevented from infiltrating the tissue any further.

A peptide or protein of the general formula II, wherein Z_5 denotes a peptide residue comprising the following amino acid sequence (SEQ ID NO 12):

Glu Arg His Gln Ser Ala Cys Lys Asp Ser Asp Trp Pro
Phe Cys Ser Asp Glu Asp Trp Asn Tyr Lys

and Z_1 denotes a proline residue,
Arg denotes an arginine residue,
Z_3 denotes a valine residue,
Z_4 denotes a valine residue,

0 1 JUN 2005

has the effect of preventing the cells of the peripheral blood from adhering to fibrin or fibrin fragments, hence prohibiting their migration in the tissue.

The described cleavage products are also known in the literature as peptide Bbeta and peptide Aalpha. Said above mentioned proadhesive and promigratory path is a completely new one for the system of controlling the migration of cells from the blood into the tissue. This function of the fibrin may be blocked by peptide Bbeta and also by peptide Aalpha.

Therefore, said peptides according to the invention are suitable as therapeutic agents for humans and animals in order to block the migration of cells from the blood into the tissue. Since fibrin or other fibrinogen products produced by proteolytic cleavage, such as, f.i., fibrinogen cleaved by an urokinase-plasminogen-activator, are generated only to a specific and regionally limited extent, i.e. at sites of inflammation, disturbed coagulation, arterial sclerosis, thrombosis and/or tumour growth, the effect of said therapeutic agent is regionally limited, which means that pathological side effects occurring in other places are not to be expected or only to a limited extent.

Preferable and completely unexpected fields of application for the peptides and/or proteins according to the invention consist in the preparation of pharmaceutical compositions for the therapy or prevention of local and/or generalized inflammations in the body in case of infectious genesis, based upon an auto-immune reaction, based upon a rheumatic disease, based upon a disorder in the immune system, based upon a genetic disease, for the prevention and/or therapy of the rejection occurring after organ transplants, of arterial sclerosis, of a reperfusion trauma, based upon arteriosclerotic and/or thrombotic diseases and an increased fibrin deposition. Such a peptide, in particular Bbeta, is also excellently suitable for the preparation of a pharmaceutical composition which accomplishes the transportation of a further drug substance to human or animal endothelial cells. In doing so, the drug substance to be transported is coupled to the peptide at one end and then, via VE-cadherin, deposits on a free spot of the vascular wall, i.e. on an endothelial cell.

In the following, the invention is explained in further detail by way of examples.

Example 1:

Preparation of the fibrinogen cleavage products:

01 JUN 2005

Non-polymerizing degradation products of fibrinogen were obtained by means of a decomposition involving cyanogen bromide according to Blombäck et al. (Nature 1968, 218; 130-134). The fibrinogen thus degraded largely consists of a 63 kD fragment, i.e. the N-terminal disulfide knot, NDSK, and comprises Aalpha-chain 1-51, Bbeta-chain 1-118 and gamma-chain 1-78. In order to obtain NDSK-II (NDSK minus fibrinopeptides A and B), the N-terminal amino acids of the Aalpha- and Bbeta-chains were cleaved off with thrombin (20 units/1μg NDSK) in three hours at room temperature and subsequently were treated with diisopropylfluorophosphate in order to block thrombin activity. The NDSK-II thus obtained consisted of Aalpha-chain 17-51, Bbeta-chain 15-118 and gamma-chain 1-78.

In order to obtain NDSK-uPA, 500 μg of NDSK was treated with 200 units of urokinase-plasminogen-activator (uPA) of Messrs. Technoclone, Vienna, Austria, for one hour at 37°C. The reaction was stopped with 5 mM phenylmethylsulfonyl fluoride. The NDSK-uPA thus obtained is a NDSK and has no fibrinopeptide B.

As a negative control, a second fraction was obtained from the fibrinogen cleavage products referred to as FCB-2 according to Nieuwenhuizen et al. (Biochem Biophys Acta 1983, 755; 531-533), which cleavage products were produced by being treated with cyanogen bromide. FCB-2 is a protein having a size of 43 kD and consists of Aalpha-chain 148-208, Bbeta-chain 191-305 and gamma-chain 95-265. For control purposes, thrombin and diisopropylfluorophosphate were added to said protein. That, however, did not result in any change to the protein (in the following, referred to as FCB-2-thr).

For the purpose of further negative controls, culture medium (RPMI of Messrs. Life techn. Inc., Paisky, UK) was treated with thrombin as above and, subsequently, was inactivated (RPMI-thr) or was treated with uPA as above and was inactivated (RPMI-uPA).

Example 2:

Peptide Aalpha (SEQ ID NO 12) corresponds to amino acids 1 to 28 of the alpha-chain of the fibrin and is identical to amino acids 17 to 45 of the Aalpha-chain of the fibrinogen:

Gly Pro Arg Val Val Glu Arg His Gln Ser Ala Cys Lys
Asp Ser Asp Trp Pro Phe Cys Ser Asp Glu Asp Trp Asn
Tyr Lys

01 JUN 2005

Peptide Bbeta (SEQ IN NO 11) corresponds to amino acids 1 to 28 of the beta-chain of the fibrin, which is identical to amino acids 15 to 43 of the Bbeta-chain of the fibrinogen, which exhibits the following sequence:

Gly	His	Arg	Pro	Leu	Asp	Lys	Lys	Arg	Glu	Glu	Ala	Pro
Ser	Leu	Arg	Pro	Ala	Pro	Pro	Pro	Ile	Ser	Gly	Gly	Gly
Tyr	Arg											

By applying a fluorenylmethyloxycarbonyl (FMOC)-protective group strategy according to Carpino L.A. and Han. G Y, J. Amer. Chem. Soc. 1981; 37; 3404-3409, both peptides were synthesized by means of a solid-phase peptide synthesis according to Merrifield R.B., J. Amer. Chem. Soc. 1963; 85, 2149-2154, using a multiple peptide synthesizer. The crude peptides were purified by preparative reversed-phase HPLC via a Nucleosil 100-10, C18-column according to Engelhart H. and Müller H. Chromatography 1984 19:77 as well as Henschen A., Hupe K.P. and Lottspeich F. High Performance Liquid Chromatography VCH 1985. As control peptides, peptides of the same length but comprising a randomized amino acid sequence were used.

Example 3:

HU-SCID mouse-model:

Human skin was transplanted onto the backs of SCID mice, and two weeks later human lymphocytes were injected into the peritoneum. The proceedings were according to Petzelbauer et al. (J. Invest. Dermatol. 1996, 107; 576-581). Then, fifteen mice thus prepared were injected in their tail veins with the following:

a) 100 µg of human NDSK-II
b) 100 µg of human FCB-2
c) 100 µg of peptide Aalpha
d) 100 µg of peptide Bbeta
e) 100 µg of randomized Aalpha
f) 100 µg of randomized Bbeta

Twenty-four hours later, the human skin was removed and the number of inflammatory sites, expressed in cells per 0.3 mm^2, was evaluated and the mean value was determined with a standard deviation.

01 JUN 2005

For a: 22 +/- 2.8
for b: 9 +/- 2.1
for c: 4 +/- 1.1
for d: 6 +/- 1.1
for e: 5 +/- 1.2
for f: 7 +/- 1.3

That allows the conclusion that NDSK-II causes inflammations, and hence said protein was used as a pathogenic substance. The other compounds per se do not exhibit any significant increase in the amount of inflammatory cells.

Comparative example 4:

Fifteen mice according to Example 3 were injected in their tail veins with

100 µg of human NDSK-II and
100 µg of randomized peptide Aalpha.

Further proceedings were according to Example 3. Per 0.3 mm², 23 +/- 3.5 inflammatory sites could be determined.

Comparative example 5:

Fifteen mice according to Example 3 were injected in their tail veins with

100 µg of human NDSK-II according to Example 1 and
100 µg of randomized peptide Bbeta.

Further proceedings were according to Example 3. Per 0.3 mm², 24 +/- 2 inflammatory sites could be determined.

Example 6:

Fifteen mice according to Example 3 were injected with

100 µg of human NDSK-II and

01 JUN 2005

100 µg of synthesized peptide Aalpha.

Further proceedings were according to Example 3. Per 0.3 mm², 21 +/- 2.2 inflammatory sites could be determined.

Example 7:

Fifteen mice according to Example 3 were injected in their tail veins with

100 µg of human NDSK-II and
100 µg of synthesized peptide Bbeta.

Further proceedings were according to Example 3. Per 0.3 mm², 14 +/- 2 inflammatory sites could be determined.

Examples 4 to 7 show that peptide Bbeta blocks lymphocytic inflammation.

Comparative example 8:

Endothelial cells from human umbilical veins (HUVEC) were marked with a red fluorescent dye (Cell Tracker Orange, 1 µl/ml, Molecular Probes, Eugene, OR) and were dispersed on a collagen matrix (Collaborative Biomedical Products, Bedford, MA). Upon confluence of the endothelial cells, peripheral mononuclear blood cells (PBMC) (10^5 cells per 25 mm^2) marked with a green fluorescent dye (Cell Tracker Green, 1 µl/ml, Molecular Probes of Messrs. Eugene, Origon) were superimposed. Thereafter, the cells were incubated at 37°C for twelve hours.

Adhering cells that had transmigrated into the gel were photographed with a laser-scan microscope, were converted into pixels and were evaluated by means of an „NIH image" according to Gröger et al. (J. Immunol. Method 1999; 222: 101-109).

It was feasible to determine the number of adherent cells per 0.1 mm² such as mentioned under „adhesion". It was feasible to determine the number of migrated cells per 0.04 mm³ such as mentioned under „migration". The mean value of three times three trials was evaluated together with the standard deviation.

0 1 JUN 2005

		adhesion	migration
a) RPMI-uPA	0.1 µg/ml	40 +/- 4	4 +/- 3
	1.0 µg/ml	38 +/- 2	5 +/- 2
	10.0 µg/ml	32 +/- 4	5 +/- 1
b) NDSK	0.1 µg/ml	31 +/- 18	6 +/- 3
	1.0 µg/ml	35 +/- 18	5 +/- 2
	10.0 µg/ml	36 +/- 24	6 +/- 3
c) NDSK-II	0.1 µg/ml	55 +/- 21	12 +/- 5
	1.0 µg/ml	67 +/- 31	19 +/- 12
	10.0 µg/ml	65 +/- 31	19 +/- 10
d) NDSK-uPA	0.1 µg/ml	58 +/- 3	10 +/- 2
	1.0 µg/ml	60 +/- 3.5	14 +/- 3
	10.0 µg/ml	65 +/- 3	18 +/- 1.5
e) FCB2	0.1 µg/ml	30 +/- 26	6 +/- 4
	1.0 µg/ml	10 +/- 10	3 +/- 2
	10.0 µg/ml	21 +/- 7	5 +/- 4
f) FCB-2-thr	0.1 µg/ml	20 +/- 12	6 +/- 5
	1.0 µg/ml	23 +/- 13	7 +/- 5
	10.0 µg/ml	26 +/- 11	4 +/- 2
g) RPMI-thr	0.1 µg/ml	29 +/- 15	4 +/- 5
	1.0 µg/ml	26 +/- 14	5 +/- 5
	10.0 µg/ml	41 +/- 20	5 +/- 4

That allows the conclusion that NDSK-II results in significant migrations of peripheral blood-monocellular cells (PBMC) to a greater extent than NDSK-uPA and hence exhibits pathogenic activity. None of the controls a), b), e), f) and g) resulted in any significant migration.

01 JUN 2005

Example 9:

100 µg of NDSK-II and Bbeta or Bbeta randomized were added to the collagen matrix according to Example 8 comprising the suspension of PBMC, and further proceedings were in accordance with Example 8.

		adhesion	migration
a)	no addition of NDSK-II	38 +/- 15	6 +/- 4
b)	only 100 µg of NDSK-II	73 +/- 29	16 +/- 7
c)	10 µg of Bbeta + NDSK-II	63 +/- 33	7 +/- 4
d)	100 µg of Bbeta + NDSK-II	47 +/- 34	5 +/- 4
e)	1000 µg of Bbeta + NDSK-II	52 +/- 27	10 +/- 6
f)	10 µg of Bbeta randomized + NDSK-II	77 +/- 33	16 +/- 6
g)	100 µg of Bbeta randomized + NDSK-II	86 +/- 35	15 +/- 6
h)	1000 µg of Bbeta randomized + NDSK-II	78 +/- 31	13 +/- 8

As can be gathered from those test results, peptide Bbeta blocks inflammations.

Example 10:

100 µg of NDSK-II and Aalpha or Aalpha randomized were added to the collagen matrix according to Example 8 comprising the suspension of PBMC, and further proceedings were in accordance with Example 8.

		adhesion	migration
a)	no addition of NDSK-II	42 +/- 6	10 +/- 1
b)	only NDSK-II	96 +/- 11	24 +/- 3
c)	10 µg of Aalpha + NDSK-II	69 +/- 12	21 +/- 4
d)	100 µg of Aalpha + NDSK-II	73 +/- 13	15 +/- 6
e)	1000 µg of Aalpha + NDSK-II	70 +/- 6	13 +/- 5
f)	10 µg of Aalpha randomized + NDSK-II	70 +/- 6	25 +/- 2
g)	100 µg of Aalpha randomized + NDSK-II	65 +/- 16	24 +/- 3
h)	1000 µg of Aalpha randomized + NDSK-II	70 +/- 12	26 +/- 3

As can be gathered from the test results, peptide Aalpha blocks the migration of PBMC only partially.

01 JUN 2005

Example 11:

Since PBMC substantially consists of a mixture of lymphocytes and monocytes, pure lymphocytes instead of PBMC (as in Examples 8-10) were used in Example 11.

100 µg of NDSK-uPA or 100 µg of NDSK-II, respectively, and Aalpha or Bbeta, respectively, were added to the collagen matrix according to Example 8 comprising endothelial cells and lymphocytes.

		adhesion	migration
a)	no addition	68 +/- 8	16 +/- 3
b)	NDSK-uPA	143 +/- 11	53 +/- 5
c)	NDSK-II	119 +/- 11	43 +/- 4
d)	only 100 µg of Bbeta	58 +/- 18	14 +/- 1
e)	NDSK-uPA + 100 µg of Bbeta	74 +/- 8	19 +/- 2
f)	NDSK-II + 100 µg of Bbeta	74 +/- 8	17 +/- 3
g)	only 100 µg of Aalpha	77 +/- 4	18 +/- 1
h)	NDSK-uPA + 100 µg of Aalpha	131 +/- 4	40 +/- 3
i)	NDSK-II + 100 µg of Aalpha	131 +/- 4	44 +/- 4
j)	only 100 µg of Bbeta randomized	75 +/- 5	19 +/- 1
k)	NDSK-uPA + 100 µg of Bbeta randomized	134 +/- 13	46 +/- 4
l)	NDSK-II + 100 µg of Bbeta randomized	120 +/- 12	42 +/- 4

Those test results show

1) that both NDSK-II and NDSK-uPA promote lymphocytic inflammation,

2) that peptide Bbeta completely blocks the lymphocytic adhesion and migration induced by NDSK-II and NDSK-uPA, whereas peptide Aalpha exhibits no blocking activity, which suggests that the free alpha-chain is not required for inducing the adhesion and migration of the lymphocytes.

Example 12:

The proceedings were in accordance with Example 11, except for pure monocytes being used instead of lymphocytes. 100 µg of NDSK-uPA or 100 µg of NDSK-II, respectively, was added to peptide Aalpha, randomized Aalpha, Bbeta or randomized Bbeta.

01 JUN 2005

		adhesion	migration
a)	no addition	43 +/- 8	7 +/- 1
b)	NDSK-uPA	48 +/- 10	10 +/- 2
c)	NDSK-II	90 +/- 11	19 +/- 6
d)	100 µg of Bbeta	59 +/- 7	5 +/- 1
e)	NDSK-uPA + 100 µg of Bbeta	61 +/- 11	8 +/- 3
f)	NDSK-II + 100 µg of Bbeta	70 +/- 7	7 +/- 5
g)	100 µg of Bbeta randomized	40 +/- 7	6 +/- 1
h)	NDSK-uPA + 100 µg of Bbeta randomized	45 +/- 5	8 +/- 3
g)	NDSK-II + 100 µg of Bbeta randomized	92 +/- 10	20 +/- 7
j)	100 µg of Aalpha	59 +/- 6	5 +/- 1
k)	NDSK-uPA + 100 µg of Aalpha	62 +/- 4	8 +/- 5
l)	NDSK-II + 100 µg of Aalpha	68 +/- 10	9 +/- 6
m)	100 µg of Aalpha randomized	58 +/- 7	6 +/- 1
n)	NDSK-uPA + 100 µg of Aalpha randomized	50 +/- 10	10 +/- 4
o)	NDSK-II + 100 µg of Aalpha randomized	108 +/- 8	21 +/- 5

Those test results show that only NDSK-II and not NDSK-uPA promotes the migration of monocytes, which means that both the alpha-chain and the beta-chain have to exhibit a free N-terminal end and block the migration of the monocytes.

Example 13:

The proceedings were in accordance with Example 11, with pure lymphocytes being used. 100 µg of NDSK-uPA or 100 µg of NDSK-II, respectively, was added to the short peptide salts derived from Aalpha Gly Pro Arg (Pro)-NH_2 acetate (Aalpha derivative) or derived from Bbeta Gly His Arg Pro-OH acetate (Bbeta derivative).

		adhesion	migration
a)	no addition	60 +/- 8	14 +/- 1
b)	NDSK-uPA	149 +/- 12	57 +/- 5
c)	NDSK-II	121 +/- 11	48 +/- 7
d)	only 100 µg of Bbeta derivative	58 +/- 10	12 +/- 9
e)	NDSK-uPA + 100 µg of Bbeta derivative	70 +/- 8	16 +/- 3
f)	NDSK-II + 100 µg of Bbeta derivative	69 +/- 7	14 +/- 5
g)	only 100 µg of Aalpha derivative	77 +/- 4	18 +/- 1

0 1 JUN 2005

h)	NDSK-uPA + 100 µg of Aalpha derivative	134 +/- 4	48 +/- 5
i)	NDSK-II + 100 µg of Aalpha derivative	131 +/- 7	49 +/- 6
j)	only 100 µg of Bbeta derivative randomized	70 +/- 5	14 +/- 7
k)	NDSK-uPA + 100 µg of Bbeta derivative randomized	130 +/- 12	49 +/- 6
l)	NDSK-II + 100 µg of Bbeta derivative randomized	120 +/- 10	55 +/- 8

Said experiment allows the conclusion that, if lymphocytic migration is inhibited, those short peptides, added continuously in an appropriate manner, exhibit the same activity as do the long peptides.

Example 14:

The proceedings were in accordance with Example 12, with pure monocytes being used. 100 mg of NDSK-uPA or 100 µg of NDSK-II, respectively, was added to the short peptide salts Aalpha Gly Pro Arg (Pro)-NH_2 acetate (Aalpha derivative) or Bbeta Gly His Arg Pro-OH acetate (Bbeta derivative).

		adhesion	migration
a)	no addition	40 +/- 8	5 +/- 1
b)	NDSK-uPA	54 +/- 9	7 +/- 2
c)	NDSK-II	85 +/- 11	22 +/- 6
d)	100 µg of Bbeta derivative	52 +/- 7	6 +/- 1
e)	NDSK-uPA + 100 µg of Bbeta derivative	61 +/- 11	8 +/- 3
f)	NDSK-II + 100 µg of Bbeta derivative	68 +/- 7	8 +/- 4
g)	100 µg of Bbeta derivative randomized	40 +/- 7	6 +/- 1
h)	NDSK-uPA + 100 µg of Bbeta derivative randomized	44 +/- 6	8 +/- 2
i)	NDSK-II + 100 µg of Bbeta derivative randomized	92 +/- 10	23 +/- 7
j)	100 µg of Aalpha derivative	50 +/- 5	4 +/- 4
k)	NDSK-uPA + 100 µg of Aalpha derivative	60 +/- 5	7 +/- 6
l)	NDSK-II + 100 µg of Aalpha derivative	64 +/- 11	8 +/- 2
m)	100 µg of Aalpha derivative randomized	54 +/- 10	6 +/- 3
n)	NDSK-uPA + 100 µg of Aalpha derivative randomized	50 +/- 10	10 +/- 4

0 1 JUN 2005

o) NDSK-II + 100 µg of Aalpha derivative randomized 99 +/- 8 21 +/- 7

Said experiment allows the conclusion that, if monocytic migration is inhibited, those short peptides, added continuously in an appropriate manner, exhibit the same activity as do the long peptides.

Example 15:

The tests were carried out on male wistar rats weighing between 220 g and 280 g. The rats were given standard food and water. For carrying out the test, the rats were anaesthetized and artifically respirated with a frequency of 70 pulses per minute, whereby from 8 ml to 10 ml per kilogram of a gas containing 30 % by volume of oxygen and having an overpressure of from 1 mm to 2 mm mercury was emitted. The cardiac artery on the right hand side was equipped with a measuring cannula, and the blood pressure in the artery as well as the heartbeats were determined. The pressure rate was determined as a product of the blood pressure in the artery and of the heartbeat rate with the dimension mm mercury / minute / 10^3. The vein on the right hand side was equipped with a measuring cannula for doping the test substances. After carrying out the surgical treatment, 2 ml of rat blood was supplied to the heart. Thirty minutes later, the cardiac artery on the left hand side was occluded. Another twenty-five minutes later, the occlusion was released in order to resupply the ischaemic area with blood. At that point of time, 800 µg/kg of peptide Bbeta or peptide Bbeta randomized, respectively, was intravenously administered to half of the animals, and then two hours were allowed to pass.

In order to distinguish between damaged and undamaged cardiac tissue, the cardiac artery on the left hand side was then supplied with evans blue dye at a concentration of 2 % by weight. Thereupon, the removed heart was dissected by five horizontal cuts, the right hand wall of the vein was removed and the sections were treated with triphenyltetratolchloride (1% by weight) for twenty minutes at 37°C so as to be able to distinguish between normal tissue and infarct tissue. The sections were evaluated by computer-sustained planimetry.

Because of the vascular occlusion, 62.5% of the cardiac muscle in the hearts of the reference rats was threatened, as opposed to 60% in the hearts of the test rats. In the hearts of the reference rats, 46% of the endangered tissue was dead, as opposed to 29% in the hearts of the test rats. That corresponds to a 37% reduction of dead tissue ($p<0.05$).

The substances according to the invention as well as the use of the substances according to the invention for preparing a pharmaceutical composition are of special significance:

For a pharmaceutical composition used in the therapy of diseases caused by the tissue-damaging effect of autoreactive lymphocytes.

Among those are diseases fitting into the sphere of autoimmunity, such as collagenoses, rheumatic diseases, psoriasis and post-/parainfectious diseases and diseases caused by a graft versus host reaction. A healing effect occurs, since said pharmaceutical composition blocks the migration of lymphocytes into the tissue. Thus, the lymphocytes remain in the bloodstream and are incapable of producing an autoreactive tissue-damaging effect.

A healing effect occurs with a drug for the therapy and/or prevention of the rejection occurring after organ transplants, since said drug prevents the migration of lymphocytes from the bloodstream into the foreign organ and hence the foreign organ cannot be destroyed by autoreactive lymphocytes.

A healing effect occurs with a drug for the therapy and/or prevention of arterial sclerosis after organ transplants, since said drug prohibits the migration of lymphocytes and monocytes into the vascular wall and hence prevents the activation of the cells of the vascular wall. In doing so, the occurrence of arterial sclerosis following organ transplants is minimized or prevented.

A healing effect occurs with a drug for the therapy and/or prevention of a reperfusion trauma following a surgically or pharmaceutically induced restoration of the blood flow such as, f.i. after cardiac infarction, apoplectic stroke, after vascular surgery, bypass surgery and organ transplants, since said drug inhibits the migration of lymphocytes and monocytes into the vascular wall. The reperfusion trauma is caused by oxygen deficiency/acidosis occurring in the cells of the vessel during the restoration of the blood flow and leads to their activation. Thereby, lymphocytes and monocytes adhere to the vascular wall and migrate into the same. The fact that lymphocytes and monocytes are prevented from adhering to and migrating into the vascular wall brings about a decrease in the hypoxia/acidosis-induced damage, without any permanent vascular damage being caused by the subsequent inflammatory reaction.

A healing effect occurs with a drug for the therapy and/or prevention of arterial sclerosis following metabolic diseases or ageing processes, since said drug inhibits the migration of

0 1 JUN 2005

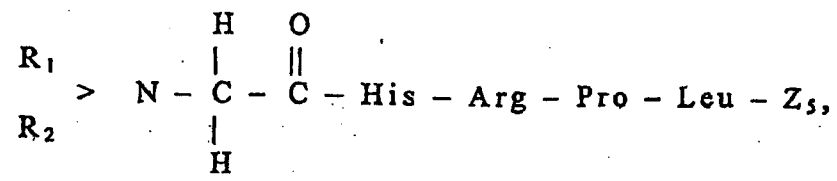
lymphocytes and monocytes into the vascular wall and hence inhibits the progredience of the arteriosclerotic plaque resulting therefrom.

The pharmaceutical composition according to the invention may also be used for transporting a further drug substance. The pharmaceutical composition according to the invention specifically binds a surface molecule to endothelial cells. Thus, drug substances coupled thereto may be contacted with endothelial cells at high concentrations, without them being able to trigger side reactions in other places. The use of substances inhibiting cell division may be mentioned as an example, which substances may exhibit an antiangiogenetic effect after having been adducted specifically to endothelial cells. In that case, tumour patients experience a healing effect, since the growth of the tumour is blocked by preventing the proliferation of endothelial cells and hence by avoiding neoangiogenesis.

01 JUN 2005

Claims:

1. The use of peptides or proteins of the general formula II (SEQ ID NO 3 to 10)



wherein R_1 and R_2, being equal or different, denote hydrogen, a saturated or unsaturated hydrocarbon residue comprising from 1 to 3, in particular up to 10, carbon atoms,

Z_5 denotes a protein residue or a peptide residue, in particular comprising from 2 to 30 amino acids,
or an alcohol residue comprising from 1 to 3, in particular up to 10, carbon atoms,
or an organic or inorganic base residue,

as well as the salts thereof, and, f.i., also amides, or mixtures with each other and/or with at least one further substance for the preparation of a pharmaceutical composition for therapeutic and/or preventive use against inflammations in human and/or veterinary medicine.

2. The use of peptides or proteins of the general formula II according to claim 1, wherein Z_5 denotes a peptide residue comprising the following amino acid sequence (SEQ ID NO 11):

Asp	Lys	Lys	Arg	Glu	Glu	Ala	Pro	Ser	Leu	Arg	Pro
Ala	Pro	Pro	Pro	Ile	Ser	Gly	Gly	Gly	Tyr	Arg	

for the preparation of a pharmaceutical composition for therapeutic and/or preventive use against inflammations in human and/or veterinary medicine.

3. The use of peptides and/or proteins according to claim 1 or 2 for the preparation of a pharmaceutical composition for the therapy of local and/or generalized inflammations in the body that are of infectious genesis.

4. The use of peptides and/or proteins according to claim 1, 2 or 3 for the preparation of a pharmaceutical composition for the therapy of local and/or generalized inflammations in the body, based upon an auto-immune reaction.

5. The use of peptides and/or proteins according to any of claims 1 to 4 for the preparation of a pharmaceutical composition for the therapy of local and/or generalized inflammations in the body, based upon a rheumatic disease.

6. The use of peptides and/or proteins according to any of claims 1 to 5 for the preparation of a pharmaceutical composition for the therapy of local and/or generalized inflammations in the body, based upon a disorder in the immune system.

7. The use of peptides and/or proteins according to any of claims 1 to 6 for the preparation of a pharmaceutical composition for the therapy of local and/or generalized inflammations in the body, based upon a genetic disease.

8. The use of peptides and/or proteins according to any of claims 1 to 7 for the preparation of a pharmaceutical composition for the prevention and/or therapy of the rejection occurring after organ transplants, of arterial sclerosis, of a reperfusion trauma, of arteriosclerotic diseases and/or of an increased fibrin deposition during the ageing process.

9. The use of peptides and/or proteins according to any of claims 1 to 8 for the preparation of a pharmaceutical composition for the prevention and/or therapy of arterial sclerosis after organ transplants.

10. The use of peptides and/or proteins according to any of claims 1 to 9 for the preparation of a pharmaceutical composition for the prevention and/or therapy of a reperfusion trauma.

11. The use of peptides and/or proteins according to any of claims 1 to 10 for the preparation of a pharmaceutical composition for the prevention and/or therapy of arteriosclerotic and/or thrombotic diseases.

01 JUN 2005

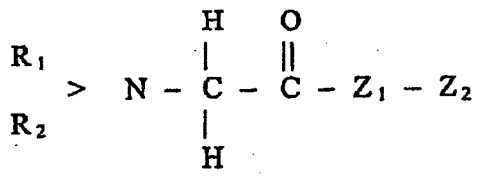
12. The use of peptides and/or proteins according to any of claims 1 to 11 for the preparation of a pharmaceutical composition for the prevention and/or therapy of an increased fibrin deposition during the ageing process.

13. The use of peptides and/or proteins according to any of claims 1 to 12 for the preparation of a pharmaceutical composition used for transporting a further drug substance to human or animal endothelial cells.

14. A pharmaceutical composition containing at least one peptide and/or protein according to any of claims 1 to 13.

15. A pharmaceutical composition according to any of claims 1 to 14, provided in a form suitable for injection.

16. The use of peptides or proteins of the general formula I (SEQ ID NO 1, 2)



wherein R_1 and R_2, being equal or different, denote hydrogen, a saturated or unsaturated hydrocarbon residue comprising from 1 to 3, in particular up to 10, carbon atoms,

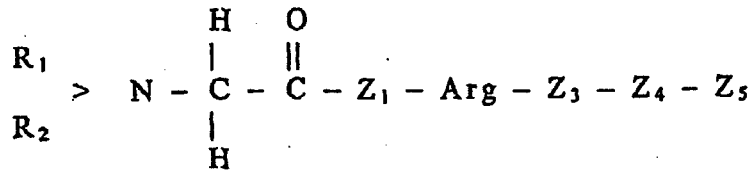
Z_1 denotes a histidine or proline residue,

Z_2 denotes an arginine residue, a peptide residue or a protein residue comprising an initial arginine residue, in particular comprising from 2 to 30 amino acids,

as well as the salts thereof, and, f.i., also amides, or mixtures with each other and/or with at least one further substance for the preparation of a pharmaceutical composition for therapeutic and/or preventive use in human and/or veterinary medicine with the purpose of preventing the adhesion of cells from the bloodstream to endothelial cells of the vascular wall and/or their subsequent transmigration from the blood into a tissue.

17. The use of peptides or proteins according to claim 16, of the general formula II (SEQ ID NO 3 to 10)

01 JUN 2005



wherein R_1 and R_2, being equal or different, denote hydrogen, a saturated or unsaturated hydrocarbon residue comprising from 1 to 3, in particular up to 10, carbon atoms,

Z_1 denotes a histidine or proline residue,
Arg denotes an arginine residue
Z_3 denotes a proline or valine residue,
Z_4 denotes a leucine or valine residue,
Z_5 denotes a protein residue or a peptide residue, in particular comprising from 2 to 30 amino acids,
or an alcohol residue comprising from 1 to 3, in particular up to 10, carbon atoms,
or an organic or inorganic base residue,

as well as the salts thereof, and, f.i., also amides, or mixtures with each other and/or with at least one further substance for the preparation of a pharmaceutical composition for therapeutic and/or preventive use in human and/or veterinary medicine with the purpose of preventing the adhesion of cells from the bloodstream to endothelial cells of the vascular wall and/or their subsequent transmigration from the blood into a tissue.

18. The use of peptides or proteins of the general formula II according to claim 17, wherein Z_5 denotes a peptide residue comprising the following amino acid sequence (SEQ ID NO 11):

Asp	Lys	Lys	Arg	Glu	Glu	Ala	Pro	Ser	Leu	Arg	Pro
Ala	Pro	Pro	Pro	Ile	Ser	Gly	Gly	Gly	Tyr	Arg	

and Z_1 denotes a histidine residue,
Arg denotes an arginine residue,
Z_3 denotes a proline residue,
Z_4 denotes a leucine residue

01 JUN 2005

for the preparation of a pharmaceutical composition for therapeutic and/or preventive use in human and/or veterinary medicine with the purpose of preventing the adhesion of cells from the bloodstream to endothelial cells of the vascular wall and/or their subsequent transmigration from the blood into a tissue.

19. The use of peptides or proteins according to claim 17 of the general formula II, wherein Z_5 denotes a peptide residue comprising the following amino acid sequence (SEQ ID NO 12):

Glu	Arg	His	Gln	Ser	Ala	Cys	Lys	Asp	Ser	Asp	Trp
Pro	Phe	Cys	Ser	Asp	Glu	Asp	Trp	Asn	Tyr	Lys	

and Z_1 denotes a proline residue,
Arg denotes an arginine residue,
Z_3 denotes a valine residue,
Z_4 denotes a valine residue

for the preparation of a pharmaceutical composition for therapeutic and/or preventive use in human and/or veterinary medicine with the purpose of preventing the adhesion of cells from the bloodstream to endothelial cells of the vascular wall and/or their subsequent transmigration from the blood into a tissue.

20. A pharmaceutical composition containing at least one peptide and/or protein according to any of claims 16 to 19.

21. A pharmaceutical composition according to any of claims 16 to 20, provided in a form suitable for injection.

01 JUN 2005

SEQUENCE LISTING

<110> FIBREX Medical Research & Development GesmbH
Petzelbauer, Peter

<120> Peptides and/or proteins as well as their use for preparing a therapeutic and/or preventive pharmaceutical composition

<130> 2760 PCT
<140> PCT/AT 01/00387
<141> 2001-12-07
<150> AT A 2063/2000
<151> 2000-12-12
<160> 2
<170> PatentIn Version 3.1

<210> 1
<211> 28
<212> PRT

<213> artificial sequence;

<220>
<221> source
<223> /note = „corresponds to amino acids 1-28 having the sequence of the Bbeta-chain of the fibrin"
<400> 1

Gly His Arg Pro Leu Asp Lys Lys Arg Glu Glu Ala Pro Ser Leu Arg
1 5 10 15
Pro Ala Pro Pro Pro Ile Ser Gly Gly Gly Tyr Arg
20 25

<210> 2
<211> 28
<212> PRT

01 JUN 2005

<213> artificial sequence

<220>

<221> source

<223> /note = „corresponds to amino acids 1-28 having the sequence of the Aalpha-chain of the fibrin"

<400> 2

Gly	Pro	Arg	Val	Val	Glu	Arg	His	Gln	Ser	Ala	Cys	Lys	Asp	Ser	Asp
1				5					10						15
Trp	Pro	Phe	Cys	Ser	Asp	Glu	Asp	Trp	Asn	Tyr	Lys				
			20					25							