

(19) **DANMARK**



Patent- og
Varemærkestyrelsen

(10) **DK/EP 2861241 T3**

(12) **Oversættelse af
europæisk patentskrift**

-
- (51) Int.Cl.: **A 61 K 38/08 (2019.01)** **C 07 K 7/06 (2006.01)** **C 07 K 14/705 (2006.01)**
- (45) Oversættelsen bekendtgjort den: **2021-07-05**
- (80) Dato for Den Europæiske Patentmyndigheds bekendtgørelse om meddelelse af patentet: **2021-04-28**
- (86) Europæisk ansøgning nr.: **13729753.7**
- (86) Europæisk indleveringsdag: **2013-06-19**
- (87) Den europæiske ansøgnings publiceringsdag: **2015-04-22**
- (86) International ansøgning nr.: **EP2013062806**
- (87) Internationalt publikationsnr.: **WO2013190014**
- (30) Prioritet: **2012-06-19 EP 12305697**
- (84) Designerede stater: **AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR**
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- (54) Benævnelse: **FORBEDREDE CD31-PEPTIDER**
- (56) Fremdragne publikationer:
WO-A1-2010/000741
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Fortsættes ...

DESCRIPTION

Field of the invention

[0001] The present invention discloses synthetic peptides corresponding and related to fragments of CD31 that favour endothelial physiologic functions while inhibiting platelet and leukocyte activation, and to their use in the treatment of disease. These peptides find use in treatment of inflammatory and thrombotic diseases, in particular when immobilised onto medical devices in contact with body fluids.

Background

Thrombotic disorders

[0002] In a healthy person, a homeostatic balance exists between procoagulant (clotting) forces and anticoagulant and fibrinolytic forces. Numerous genetic, acquired, and environmental factors can tip the balance in favor of coagulation, leading to the pathologic formation of thrombi in veins (e.g. deep vein thrombosis), arteries (e.g. atherothrombosis, myocardial infarction, ischemic stroke), or cardiac chambers. Thrombi can obstruct blood flow at the site of formation or detach and embolize to block a distant blood vessel (e.g. pulmonary embolism, stroke).

[0003] Accumulating evidence show that atherothrombosis, a world-leading life-threatening disease, is linked to inappropriate activation of blood leukocytes and platelets leading to a destructive inflammatory response within the vascular wall and thrombotic occlusion and/or rupture due the fibrinolytic cascade. Consequently, a restoration of the physiologic cell regulation at the interface between the blood and the vessel wall would represent an innovative therapeutic option to fight atherothrombosis.

Inflammatory and autoimmune disorders

[0004] Inflammatory disorders underlie a large number of human diseases. The immune system is often involved with inflammatory disorders, demonstrated in both allergic reactions and some myopathies, with many immune system disorders resulting in abnormal inflammation. Non-immune diseases with etiological origins in inflammatory processes include cancer, atherosclerosis, and ischaemic heart disease.

[0005] In autoimmune disorders, the immune system produces antibodies to an endogenous

antigen. Antibody-coated cells, like any similarly coated foreign particle, activate the complement system, resulting in tissue injury. Autoimmune disorders include systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), multiple sclerosis (MS), inflammatory bowel disease (IBD), Graves' disease and diabetes mellitus. Systemic vasculitis, venous chronic insufficiency and atherosclerosis-related vascular diseases are also comprised within autoimmune-inflammatory disorders in which the target tissue is the vascular wall and the nearby organs and tissues.

[0006] Several mechanisms may account for the body's attack on itself. Autoantigens may become immunogenic because they are altered chemically, physically, or biologically. Certain chemicals couple with body proteins, making them immunogenic (as in contact dermatitis). Drugs can produce several autoimmune reactions by binding covalently to serum or tissue proteins (see below). Photosensitivity exemplifies physically induced autoallergy: Ultraviolet light alters skin protein, to which the patient becomes allergic. In animal models, persistent infection with an RNA virus that combines with host tissues alters autoantigens biologically, resulting in an autoallergic disorder resembling SLE.

[0007] Most human autoimmune diseases are driven by antigen-specific, adaptive immune cells (T- and B-cell lymphocytes). T- and B-cell clones responding to specific antigenic epitopes are responsible for the initiation and/or the propagation of these diseases. Similarly, specific antigen-driven T- and B-cell responses are responsible for the rejection of organ allografts. In addition to the adaptive immune cells, cells of the innate immune system, which are not specific of a given antigen, are also involved in the pathogenesis of chronic inflammatory (autoimmune) disorders, such as granulocytes, monocyte-macrophages, dendritic cells and natural killer cells.

CD31 (PECAM-1)

[0008] CD31 is a single chain, homophilic transmembrane receptor exclusively and constitutively present on all endothelial cells, platelets and leukocytes. CD31 consists of a single chain 130-kDa glycoprotein comprising six Ig-like extracellular domains, a short transmembrane segment and a cytoplasmic tail. The cytoplasmic tail contains two important tyrosine- based motifs (around Y663 and Y686) that form specific docking sites for SH2-containing adaptor molecules that preferentially associate with phosphatases and act as ImmunoTyrosine-based Inhibitory Motif (ITIM)s. The structure of CD31 is shown in the table below.

Domain	Position on SEQ ID No: 1
Signal peptide	1 to 27
Extracellular domain	28 to 601
First Ig-like extracellular domain	34 to 121
Second Ig-like extracellular domain	145 to 233

Domain	Position on SEQ ID No: 1
Third Ig-like extracellular domain	236 to 315
Fourth Ig-like extracellular domain	328 to 401
Fifth Ig-like extracellular domain	424 to 493
Sixth Ig-like extracellular domain	499 to 591
Juxta-membrane domain	592 to 601
Transmembrane domain	602 to 620
Cytoplasmic domain	621 to 738

[0009] The intracellular CD31 ITIMs are not phosphorylated in resting conditions because CD31 does not possess an intrinsic kinase activity. CD31 molecules bind to each other via a trans-homophilic liaison of the Ig-like domains 1-2 between interacting cells. This trans-homophilic binding is required to trigger the clustering of the CD31 molecules on the membrane plane, which in turn requires a cis-homophilic juxtamembrane sequence. The phosphorylation of the CD31 intracellular ITIMs becomes then possible because its ITIMs can be exposed to the activity of the tyrosine kinases that are carried close by other cluster-associated membrane receptors (such for instance the T cell receptor). The phosphorylation of the intracellular ITIMs triggers the recruitment and activation of intracellular SH2-containing phosphatases. Depending on the signalling adaptors associated to the closest membrane receptor, the activation of SH2-containing phosphatases can lead to either the activation of signalling cascades (e.g. GAB/ERK/MAPK, driving adherence and growth of endothelial cells, foxp3 expression and differentiation of lymphocytes into the regulatory phenotype, driving active cell-cell detachment) or their inhibition (e.g. JAK/STAT, preventing leukocyte and platelet activation). Accordingly, the function of CD31 varies upon the cell type.

[0010] The presence of CD31 at high density at endothelial intercellular borders has previously led to the hypothesis that CD31 functions as a cell adhesion molecule in endothelial cells, involved in leukocyte extravasation. However, CD31 is excluded from the tight endothelial cell junctions and experimental evidence rather show it prevents leukocyte extravasation since the latter increases in the CD31-deficient mice. Endothelial CD31 becomes tyrosine-phosphorylated upon mechanical stress and its function is necessary to stabilize the endothelial structure and angiogenesis.

[0011] CD31 becomes tyrosine-phosphorylated following activation and aggregation of platelets. This represents a negative feedback mechanism because CD31 clustering inhibits platelet aggregation and thrombus formation by uncoupling signal transduction through several activatory receptors. The immunoregulatory properties of CD31 are supported by the fact that CD31 signalling drives mutual repulsion of blood leukocytes and modulates the balance between inhibitory and stimulatory signals of both innate and adaptive immune cells. Mechanical engagement of the distal Ig-like extracellular domains of CD31 induces outside-in inhibitory signalling triggered by the phosphorylation of its ITIMs, and the recruitment and

activation of SH2-containing phosphatases.

[0012] Zehnder et al. (1995, Blood. 85(5):1282-8) identified a CD31 antibody that inhibited the mixed lymphocyte reaction (MLR) in a specific and dose-dependent manner. They further found that a CD31 peptide corresponding to the epitope of this antibody, i.e. to the 23 membrane-proximal amino acids of CD31, strongly inhibited the MLR. They hypothesized that the 23 membrane-proximal amino acids of CD31 constitutes a functionally important region, and that the CD31 peptide interferes with lymphocyte activation by competing for binding epitopes. However, Zehnder et al. failed to teach whether CD31-mediated signaling is activated or inhibited by the CD31 peptide.

[0013] Chen et al. (1997, Blood. 89(4):1452-9) showed that this peptide delayed onset of graft-versus-host disease (GVHD) and increased long-term survival in a murine model of the disease. They hypothesized that the CD31 peptide inhibits a common pathway in T-cell activation. Again, Chen et al. failed to elucidate the role played by the CD31 peptide in T-cell activation. In particular, these previous works did not assess the putative effect of the peptide on the CD31 signaling cascade and more precisely on the phosphorylation state of the CD31 ITIMs.

[0014] By a yet unknown mechanism, CD31 is "lost" on certain circulating lymphocytes. Its loss is observed upon lymphocyte activation and it has been recently shown that the absence of lymphocyte CD31 signalling, in turn, heightens the pathologic immune responses involved in the development of atherothrombosis.

[0015] A soluble form of CD31, due to a variant transcript lacking the transmembrane segment, has also been reported and therefore it is currently thought that the individual amount of circulating CD31 is genetically determined. Consequently, a number of previous studies have attempted to find a correlation between plasma levels of soluble CD31 and the risk of atherothrombosis or other inflammatory diseases. However, independently of the specific genetic polymorphisms analyzed, data showed a broad range of plasma CD31 values and the results of these different studies were contradicting.

[0016] There is therefore a need for better understanding the biological function of CD31. This would allow the provision of more efficient therapeutics for the treatment of diseases linked with T-cell activation.

[0017] The inventors have previously reported that the assumed loss of CD31 on activated/memory T lymphocytes is actually incomplete and results from shedding of CD31 between the 5th and the 6th extracellular Ig-like domains (described in international patent publication WO2010/000741). The shed extracellular domain of CD31 (further referred to as "shed CD31") is then released into the circulation, where it is present together with a soluble splice variant of CD31. In addition, they have shown that a high risk of atherothrombosis is linked with the increase in shed CD31 and decrease in splice variant CD31 in the circulation, and not with the total level of circulating CD31.

[0018] The finding that CD31 is not lost on blood lymphocytes but only cleaved provided a unique opportunity to rescue its physiological immunoregulatory function by targeting the residual portion of the molecule.

[0019] The inventor's previous work showed that peptides corresponding to juxta-membrane amino acids of the ectodomain of CD31 are able to rescue the physiological immunoregulatory function of CD31, even in patients having apparently lost CD31 from the surface of their circulating T lymphocytes. They demonstrated that such peptides are capable of preventing disease progression and aneurysm formation in a mouse model for atherosclerosis. The peptides have unique properties compared to soluble forms of CD31 comprising all or most Ig-like domains of CD31. Indeed, such peptides are highly homophilic since they have a K_d of 10^{-7} M, as assessed by BIAcore analysis. Hence they are able to engage CD31 signaling by bridging the membrane juxta-proximal part of extracellular CD31 that remains expressed after its cleavage, via a strong homo-oligomerization. In contrast to this, alternatively spliced soluble CD31 lacks the first 10 membrane juxta-proximal amino acids and shows weak homophilic binding with the 23-mer peptide (K_d of 17 μ M, as assessed by BIAcore analysis). Furthermore, *in vitro*, only the peptides identified by the inventors are capable of engaging the ITIM pathway downstream of the truncated isoform of CD31, and are thus capable of restoring CD31 signaling in T lymphocytes having apparently lost CD31.

[0020] Document WO2010/000741 for example discloses an isolated peptide consisting of a fragment of at least 6 amino acids of the sequence defined by amino acids 579 to 601 of human CD31 or of a corresponding sequence in a non-human mammalian CD31, wherein said peptide exerts a dose-dependent inhibition of T-cell proliferation *in vitro*. Document WO2010/000741 particularly discloses a peptide of 10 amino acids, called PepReg, and corresponding to amino acids 581-590 of the herein disclosed murine CD31 sequence SEQ ID NO: 2. Peptide PepReg is for example capable of suppressing T cell proliferation *in vitro* and of arresting disease development in the waxing phase and reducing disease extension in the plateau phase in an *in vivo* model of EAE (*Experimental Autoimmune Encephalomyelitis*).

Description of the Invention

[0021] The inventors have identified a specific 8 amino acid peptide within the membrane juxta-proximal part of extracellular CD31, which is of particular utility in inhibiting platelet and leukocyte activation and in treatment of a thrombotic or an inflammatory disorder. The fact that this sequence is soluble in water is an advantage on the pharmacological point of view. They have further shown that peptide sequences corresponding to this 8 amino-acid fragment comprising inversions, and/or unnatural aminoacids, such as D-enantiomers, also retain these activities or demonstrate improved activity. Incorporation of unnatural aminoacids in peptides intended for therapeutic use is of utility in increasing the stability of the peptide, in particular *in vivo* stability.

[0022] The present invention is defined in the claims.

[0023] The application thus discloses a peptide consisting of the amino acid sequence of amino acids 582 to 589 of murine CD31 SEQ ID NO: 2 or the amino acid sequence corresponding to this sequence in another mammalian CD31, such as a human CD31 (SEQ ID NO 1) porcine CD31 (SEQ ID NO 4) or bovine CD31 (SEQ ID NO 3). Corresponding sequences may be identified by referring to the alignment of Fig 1 or any other alignments performed as described herein. Thus, the invention also provides an isolated peptide consisting of the amino acid sequence of amino acids 593-to 600 of SEQ ID NO: 1. The application also discloses peptides consisting of the above-described sequences wherein the position of two or more amino acids is inverted or displaced. Also disclosed are peptides as described above wherein one or more amino acids are in the D-enantiomer form. Preferably, the peptide is an isolated peptide.

[0024] Preferably, the peptide disclosed herein is soluble in an organic or nonorganic solvent, such as water.

[0025] Also disclosed are peptides comprising a sequence consisting of a fragment of said the amino acid sequence of amino acids 582 to 589 of SEQ ID NO: 2 or the amino acid sequence corresponding to this sequence in another mammalian CD31, such as a human CD31 (SEQ ID NO 1) porcine CD31 (SEQ ID NO 4) or bovine CD31 (SEQ ID NO 3). Corresponding sequences may be identified by referring to the alignment of Fig 1 or any other alignments performed as described herein. A 'fragment' refers to a sequence of consecutive amino acids. For example, said fragment may be a fragment of 1, 2, 3, 4, 5, 6 or 7 amino acids.

[0026] The CD31 peptide disclosed herein may comprise a chirality change such as e.g. replacement of one or more naturally occurring amino acids (L enantiomer) with the corresponding D-enantiomers. D-enantiomers of amino acids are referred to by the same letter as their corresponding L-enantiomer, but in lower case. Thus, for example, the L-enantiomer of arginine is referred to as 'R', while the D-enantiomer is referred to as 'r'. For example, 1, 2, 3, 4, 5, 6, 7 or 8 of the amino acids in the peptide may be in the D-enantiomer form. The peptides may comprise ther modified or non-natural amino acids, as described below.

[0027] The CD31 peptide disclosed herein may comprise an inverted sequence, namely an inversion of the amino acid chain (from the C-terminal end to the N-terminal end). The entire amino acid sequence of the peptide may be inverted, or a portion of the amino acid sequence may be inverted. For example, a consecutive sequence of 2, 3, 4, 5, 6, 7 or 8 amino acids may be inverted. Reference herein to 'inverted' amino acids refers to inversion of the sequence of consecutive amino acids in the sequence. The peptide may comprise a retro-inversion in which one or more naturally-occurring amino acids (L-enantiomer) are replaced with the corresponding D-enantiomers, together with an inversion of the amino acid chain (from the C-terminal end to the N-terminal end).

[0028] The peptide of the invention may thus have the sequence:

H-RVFLAPWK-OH (SEQ ID NO 5), corresponding to the amino acid sequence of amino acids 582 to 589 of SEQ ID NO: 2; (P8F)

H-kwpalfvr-OH (SEQ ID NO 6), corresponding to the inverted sequence of amino acids 582 to 589 of SEQ ID NO: 2 in D-enantiomer form, namely a retro-inversion of said sequence; (P8RI)

H-RVILAPWK-OH (SEQ ID NO 7), corresponding to the amino acid sequence of amino acids 593-600 of SEQ ID NO: 1;

H-kwpalivr-OH (SEQ ID NO 8), corresponding to the inverted sequence of amino acids 593-600 of SEQ ID NO: 1 in D-enantiomer form, namely a retro-inversion of said sequence.

[0029] In preferred embodiments, the peptide of the invention commences with the motif RV.

[0030] Also disclosed are isolated nucleic acids encoding the peptides disclosed herein, and pharmaceutical compositions comprising said peptides, as described below.

[0031] In addition to the CD31 fragment, the peptide may optionally comprise sequences heterologous to CD31. These heterologous sequences may e.g. correspond to a carrier molecule such as the Keyhole Limpet Hemocyanin (KLH), bovine serum albumin (BSA), ovalbumin (OVA), thyroglobulin (THY) or the multiple antigenic peptide (MAP). Thus the application discloses peptides comprising a peptide disclosed herein in addition to heterologous sequence.

[0032] The sequence of CD31 peptides according to the invention is preferably derived from the sequence of human or murine CD31. However, the sequence of CD31 may be derived from any non-human mammalian CD31 sequence. Figure 1 shows an alignment between the human, murine, bovine and pig CD31 sequences. The person skilled in the art can easily identify the corresponding sequence in another non-human mammalian CD31 protein by performing a sequence alignment with the sequences shown in Figure 1. Methods for sequence alignment and determination of sequence identity are well known in the art, for example using publicly available computer software such as BioPerl, BLAST, BLAST-2, CS-BLAST, FASTA, ALIGN, ALIGN-2, LALIGN, Jaligner, matcher or Megalign (DNASTAR) software and alignment algorithms such as the Needleman-Wunsch and Smith-Waterman algorithms.

[0033] CD31 peptides disclosed herein may have the biological activity of exerting a dose-dependent inhibition of T-cell proliferation *in vitro* and/or of inhibiting the mixed-lymphocyte reaction (MLR); inhibition of platelet aggregation; inhibition of platelet activation; inhibition of thrombin generation by platelets and/or inhibition of VCAM-1 expression of endothelial cells. Their biological activity may for example be measured as described in Example 1, 2, 3 or 4 or in Zehnder et al. 1995, Blood. 85(5):1282-8, Fornasa et al. 2010, J Immunol 184: 6585-6591; Fornasa et al, 2012, Cardiovascular Research 94: 30-37.

[0034] The T-cell proliferation assay may comprise comparing the radioactivity incorporated into T-cells cultured either in the presence or in the absence of the compound to be tested. This assay may for example be performed as follows:

- providing a multi-well plate comprising complete medium supplemented with anti-CD3 antibodies;
- supplementing the wells with increasing concentrations of the compound to be tested;
- plating peripheral blood mononuclear cells (or spleen cells, or lymph node cells);
- culturing the cells for about 72 hours;
- adding (^3H) thymidine and culturing the cells for about 16 hours;
- measuring the radioactivity; and
- comparing the radioactivity measured in the presence of the compound to be tested with the radioactivity measured in the absence of said compound, and/or in the presence of a reference compound, and/or in the presence of a negative control.

[0035] Alternatively, the T-cell proliferation assay may comprise the use of carboxyfluorescein diacetate succinimidyl ester (CFSE), as described in Nature Protocols, 2007, 2: 2049-2056 and in Fornasa et al, 2012, Cardiovascular Research 94: 30-37:

- Incorporate optimum amount of the fluorescent probe (CFSE) in peripheral blood mononuclear cells (or spleen cells, or lymph node cells) in the presence of complete culture medium
- providing a multi-well plate comprising, CFSE-stained cells and the stimulus (anti-CD3 antibodies)
- supplementing the wells with increasing concentrations of the compound to be tested;
- Comparing the number of daughter cells (proliferation) based on the intensity of the fluorescent staining by flow cytometry

[0036] Alternatively, the leukocyte activation may be assessed by comparing expression levels of the early activation marker CD69 leukocytes (blood mononuclear cells or spleen cells, or lymph node cells) cultured either in the presence or in the absence of the compound to be tested. This assay may for example be performed as follows:

- providing (blood mononuclear cells or spleen cells, or lymph node cells) or purified leukocyte subpopulation ;
- stimulating said cells by addition of an appropriate stimulus (anti-CD3 purified antibodies and bone marrow derived dendritic cells; or LPS or a lectin such as ConA, PHA, PWM) ;
- culturing the cells for up to 48 hours; and
- analyzing said cells for the expression of the early activation marker CD69, e.g. by flow cytometry; and
- comparing CD69 expression in the presence of the compound to be tested.

[0037] CD31 peptides according to the invention may be prepared by any well-known procedure in the art, such as solid phase synthesis, liquid phase synthesis or genetic engineering. As a solid phase synthesis, for example, the amino acid corresponding to the C-terminus of the peptide to be synthesized is bound to a support which is insoluble in organic solvents, and by alternate repetition of reactions, one wherein amino acids with their amino groups and side chain functional groups protected with appropriate protective groups are condensed one by one in order from the C-terminus to the N-terminus, and one where the amino acids bound to the resin or the protective group of the amino groups of the peptides are released, the peptide chain is thus extended in this manner. After synthesis of the desired peptide, it is subjected to the deprotection reaction and cut out from the solid support.

[0038] The CD31 peptides of the invention may optionally comprise additional chemical modifications, optionally aimed at improving their stability and/or their bioavailability. Such chemical modifications aim at obtaining peptides with increased protection of the peptides against enzymatic degradation *in vivo*, and/or increased capacity to cross membrane barriers, thus increasing its half-life and maintaining or improving its biological activity. Any chemical modification known in the art can be employed according to the present invention. Such chemical modifications include but are not limited to:

- modifications to the N-terminal and/or C-terminal ends of the peptides such as e.g. N-terminal acylation (preferably acetylation) or desamination, or modification of the C-terminal carboxyl group into an amide or an alcohol group;
- modifications at the amide bond between two amino acids: acylation (preferably acetylation) or alkylation (preferably methylation) at the nitrogen atom or the alpha carbon of the amide bond linking two amino acids;
- modifications at the alpha carbon of the amide bond linking two amino acids such as e.g. acylation (preferably acetylation) or alkylation (preferably methylation) at the alpha carbon of the amide bond linking two amino acids;
- of the amino acid chain (from the C-terminal end to the N-terminal end);
- azapeptides, in which one or more alpha carbons are replaced with nitrogen atoms; and/or
- betapeptides, in which the amino group of one or more amino acid is bonded to the β carbon rather than the α carbon.

Polypeptides include amino acid sequences modified either by natural processes, such as post-translational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini, it will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched and

branched cyclic polypeptides may result from natural posttranslational processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, araidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidyl inositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent crosslinks, formation of cystine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination.

[0039] By an "isolated" peptide, it is intended that the peptide is not present within a living organism, e.g. within human body. However, the isolated peptide may be part of a composition or a kit. The isolated peptide is preferably purified.

[0040] The compounds of the invention may be produced by any well-known procedure in the art, including chemical synthesis technologies and recombinant technologies.

[0041] Examples of chemical synthesis technologies are solid phase synthesis and liquid phase synthesis. As a solid phase synthesis, for example, the amino acid corresponding to the C-terminus of the peptide to be synthesized is bound to a support which is insoluble in organic solvents, and by alternate repetition of reactions, one wherein amino acids with their amino groups and side chain functional groups protected with appropriate protective groups are condensed one by one in order from the C-terminus to the N-terminus, and one where the amino acids bound to the resin or the protective group of the amino groups of the peptides are released, the peptide chain is thus extended in this manner. Solid phase synthesis methods are largely classified by the tBoc method and the Fmoc method, depending on the type of protective group used. Typically used protective groups include tBoe (t-butoxycarbonyl), Cl-Z (2-chlorobenzyloxycarbonyl), Br-Z (2-bromobenzyloxycarbonyl), Bzl (benzyl), Fmoc (9-fluorenylmethoxycarbonyl), Mbh (4, 4'-dimethoxydibenzhydryl), Mtr (4-methoxy-2, 3, 6-trimethylbenzenesulphonyl), Trt (trityl), Tos (tosyl), Z (benzyloxycarbonyl) and Clz-Bzl (2, 6-dichlorobenzyl) for the amino groups; NO₂ (nitro) and Pmc (2,2, 5,7, 8-pentamethylchromane-6-sulphonyl) for the guanidino groups; and tBu (t-butyl) for the hydroxyl groups). After synthesis of the desired peptide, it is subjected to the de-protection reaction and cut out from the solid support. Such peptide cutting reaction may be carried with hydrogen fluoride or trifluoromethane sulfonic acid for the Boc method, and with TFA for the Fmoc method.

[0042] Alternatively, the peptide may be synthesized using recombinant techniques. In this case, a nucleic acid encoding a peptide according to the invention (further referred to as "a nucleic acid according to the invention") is cloned into an expression vector. The nucleic acid of the invention is preferably placed under the control of expression signals (e.g. a promoter, a terminator and/or an enhancer) allowing its expression. The expression vector is then transfected into a host cell (e.g. a human, CHO, mouse, monkey, fungal or bacterial host cell), and the transfected host cell is cultivated under conditions suitable for the expression of the

peptide.

[0043] The method of producing the peptide may optionally comprise the steps of purifying said peptide, chemically modifying said peptide, and/or formulating said peptide into a pharmaceutical composition.

[0044] Preferably, the peptide disclosed herein is soluble in an organic or nonorganic solvent, for example water or aqueous buffer such as NaCl 9g/L, PBS, Tris or Tris-phosphate. Thanks to such solubility, the peptide may be dissolved in water at a concentration of, for example, 1 micromolar, 10 micromolar, 50 micromolar, 100 micromolar, 500 micromolar, 1 mM, 50 mM, 100 mM or more.

Use of CD31 peptides for the treatment of thrombotic and inflammatory disorders

[0045] It has been found that CD31 peptides according to the invention are capable of activating CD31-mediated signaling, even in CD31⁻ (i.e. CD31^{shed}) T lymphocytes. In addition, such peptides are capable of preventing disease progression and aneurysm formation in a mouse model for atherosclerosis, and improving clinical score in a mouse model of multiple sclerosis.

[0046] The disclosure therefore also provides a peptide of the invention for use in activating CD31-mediated signaling. These peptides preferably exert a dose-dependent inhibition of T-cell proliferation *in vitro*. The activation of CD31-mediated signaling may be an *in vitro* or an *in vivo* activation. Also disclosed are methods of activating CD31-mediated signalling using the peptides of the invention, *in vitro* or *in vivo*. The *in vivo* method may be a method of treatment as defined below.

[0047] As used throughout the present specification, the term "CD31-mediated signaling" refers to a signaling pathway in which CD31 is involved. Such pathways are well known in the art and include those described e.g. in Newman and Newman (2003 Arterioscler Thromb Vasc Biol 23:953-964) and in Newton-Nash and Newman (1999. J Immunol 163:682-688).

[0048] The invention therefore also provides a peptide of the invention for use in the treatment of a thrombotic or an inflammatory disorder. These peptides preferably exert a dose-dependent inhibition of T-cell proliferation *in vitro*. The activation of CD31-mediated signaling may be an *in vitro* or an *in vivo* activation. Also disclosed are methods of treatment of a thrombotic or an inflammatory disorder using the peptides of the invention, preferably comprising administration of said peptides to an individual in need thereof.

[0049] As used throughout the present specification, the term "thrombotic disorder" includes but is not limited to atherothrombosis, atherosclerosis, acute coronary syndrome, ischemic stroke, peripheral arterial disease and abdominal aortic aneurysm.

[0050] As used throughout the present specification, the term "inflammatory disorder" includes but is not limited to chronic inflammatory diseases such as inflammatory bowel disease, psoriasis, atopic dermatitis, cerebral amyloid angiopathy, vasculitis. The term also includes autoimmune disorders, including but not limited to rheumatoid arthritis (RA), multiple sclerosis (MS), inflammatory bowel disease (IBD), systemic lupus erythematosus (SLE), Graves' disease and diabetes mellitus. Other conditions such as acute and chronic graft rejection including graft versus host disease (GVHD) and septic shock may be encompassed by the term. For example, the peptides of the invention may be used to treat septic shock (optionally in combination with antibiotic therapy, e.g. large spectrum antibiotic therapy) and in transplantation such as bone marrow, kidney, heart, liver or lung transplantation.

[0051] In a preferred embodiment of the invention, said thrombotic or inflammatory disorder is associated with a loss of CD31⁺ T lymphocyte phenotype. Indeed, it has been surprisingly found that CD31 peptides restore CD31 signaling even in individuals with a CD31⁻ T lymphocytes phenotype. Therefore, in the context of the present invention, CD31 peptides are preferably used to treat a subgroup of individuals and/or patients having a CD31⁻ T lymphocytes phenotype.

[0052] As used herein, the term "CD31⁻ T lymphocyte phenotype" is used interchangeably with the term "CD31^{shed} T lymphocyte phenotype". These terms refer to the phenotype of an individual having apparently lost CD31 on its circulating T cells when conventional prior art methods for detecting CD31, e.g. such as those described in Stockinger et al. (Immunology, 1992, 75(1):53-8), Demeure et al. (Immunology, 1996, 88(1):110-5), Caligiuri et al. (Arterioscler Thromb Vasc Biol, 2005, 25(8):1659-64) or Caligiuri et al. (Arterioscler Thromb Vasc Biol, 2006, 26(3):618-23) are used. In such methods, the antibody used for detecting CD31 binds to an epitope located on any one of the 1st to the 5th extracellular Ig-like domains.

[0053] Preferably, individuals having a CD31⁻ T lymphocyte phenotype, meaning that at least 50%, 60%, 65%, 70%, 75%, 80%, 90% or 95% of their circulating T lymphocytes are CD31^{shed} lymphocytes. Either the plasma concentration of T-cell-derived truncated CD31 or the frequency of CD31⁻ T lymphocytes, compared to CD31⁺ T lymphocytes, may be measured.

[0054] The application also discloses a method of treating or preventing a thrombotic or an inflammatory disorder comprising the step of administering an effective amount of a peptide as described herein, or a nucleic coding therefore, to an individual in need thereof. Said individual in need thereof preferably suffers from or is at risk of suffering from a thrombotic or an inflammatory disorder. Most preferably, said individual has a CD31⁻ T lymphocytes phenotype.

[0055] By "effective amount", is meant an amount sufficient to achieve a concentration of peptide, which is capable of preventing, treating or slowing down the disease to be treated. Such concentrations can be routinely determined by those of skilled in the art. The amount of

the compound actually administered will typically be determined by a physician, in the light of the relevant circumstances, including the condition to be treated, the chosen route of administration, the actual compound administered, the age, weight, and response of the individual patient, the severity of the patient's symptoms, and the like. It will also be appreciated by those of skill in the art that the dosage may be dependent on the stability of the administered peptide.

[0056] The individuals to be treated in the frame of the invention are preferably human individuals. However, the veterinary use of CD31 peptides for treating other mammals is also contemplated by the present invention.

Pharmaceutical compositions

[0057] The CD31 peptides described herein may be formulated into a pharmaceutical composition. Thus the application discloses a pharmaceutical composition comprising any one of the above CD31 peptides and a physiologically acceptable carrier. Physiologically acceptable carriers can be prepared by any method known by those skilled in the art.

[0058] Pharmaceutical compositions comprising at least one peptide disclosed herein include all compositions wherein the peptide(s) are contained in an amount effective to achieve the intended purpose. In addition, the pharmaceutical compositions may contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries, which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Suitable pharmaceutically acceptable vehicles are well known in the art and are described for example in Remington's Pharmaceutical Sciences (Mack Publishing Company, Easton, USA, 1985), which is a standard reference text in this field. Pharmaceutically acceptable vehicles can be routinely selected in accordance with the mode of administration, solubility and stability of the peptides. For example, formulations for intravenous administration may include sterile aqueous solutions which may also contain buffers, diluents and other suitable additives. The use of biomaterials and other polymers for drug delivery, as well the different techniques and models to validate a specific mode of administration, are disclosed in literature.

[0059] The peptides of the present invention may be administered by any means that achieve the intended purpose. For example, administration may be achieved by a number of different routes including, but not limited to subcutaneous, intravenous, intradermal, intramuscular, intraperitoneal, intracerebral, intrathecal, intranasal, oral, rectal, transdermal, buccal, topical, local, inhalant or subcutaneous use.

[0060] The peptides of the present invention may be attached to a solid support, such as a stent. For example, the peptides may be covalently grafted onto a polymer or directly onto an aminated metal surface for use on intravascular prostheses such as stents, arterial tubes and mechanical valves. The immobilised peptide would prevent platelet and leukocyte adherence and activation to the prosthesis while promoting the acceptance/integration of the device in the

body by favouring endothelial cell attachment and growth.

[0061] The latter use could include endovascular prostheses, such as tubes and stents, artificial heart valves, bone and dental prostheses

[0062] Dosages to be administered depend on individual needs (which may be quantified by measuring cell-specific truncated CD31 in the plasma with using our bead-based method), on the desired effect and the chosen route of administration. It is understood that the dosage administered will be dependent upon the age, sex, health, and weight of the recipient, concurrent treatment, if any, frequency of treatment, and the nature of the effect desired. The total dose required for each treatment may be administered by multiple doses or in a single dose.

[0063] Depending on the intended route of delivery, the compounds may be formulated as liquid (e.g., solutions, suspensions), solid (e.g., pills, tablets, suppositories) or semisolid (e.g., creams, gels) forms or immobilised on the surface of a medical device.

[0064] In a preferred embodiment, the compositions are presented in unit dosage forms to facilitate accurate dosing. The term "unit dosage forms" refers to physically discrete units suitable as unitary dosages for human subjects and other mammals, each unit containing a pre-determined quantity of active material calculated to produce the desired therapeutic effect, in association with a suitable pharmaceutical excipient. Typical unit dosage forms include pre-filled, pre-measured ampoules or syringes of the liquid compositions or pills, tablets, capsules or the like in the case of solid compositions. In such compositions, the compound of the invention is usually a minor component (from about 0.1 to about 50% by weight or preferably from about 1 to about 40% by weight) with the remainder being various vehicles or carriers and processing aids helpful for forming the desired dosing form.

[0065] The compounds of this invention can also be administered in sustained release forms or from sustained release drug delivery systems.

[0066] The expression "physiologically acceptable" is meant to encompass any carrier, which does not interfere with the effectiveness of the biological activity of the active ingredient and that is not toxic to the host to which is administered. For example, for parenteral administration, the above active ingredients may be formulated in unit dosage form for injection in vehicles such as saline, dextrose solution, serum albumin and Ringer's solution.

[0067] Besides the pharmaceutically acceptable carrier, the compositions of the invention can also comprise minor amounts of additives, such as stabilizers, excipients, buffers and preservatives.

[0068] The application also discloses a pharmaceutical composition comprising a nucleic acid encoding the peptide of the invention in the frame of e.g. a treatment by gene therapy. In this case, the nucleic acid is preferably present on a vector, on which the sequence coding for the

peptide is placed under the control of expression signals (e.g. a promoter, a terminator and/or an enhancer) allowing its expression. The vector may for example correspond to a viral vector such as an adenoviral or a lentiviral vector.

[0069] The application further discloses kits comprising a pharmaceutical composition comprising a CD31 peptide disclosed herein and instructions regarding the mode of administration. These instructions may e.g. indicate the medical indication, and/or the route of administration, and/or the dosage, and/or the group of patients to be treated.

Prevention and treatment

[0070] 'Treatment' includes both therapeutic treatment and prophylactic or preventative treatment, wherein the object is to prevent or slow down the targeted pathologic condition or disorder. Those in need of treatment include those already with the disorder as well as those prone to have the disorder or those in whom the disorder is to be prevented. The terms 'therapy', 'therapeutic', 'treatment' or 'treating' include reducing, alleviating or inhibiting or eliminating the symptoms or progress of a disease, as well as treatment intended to reduce, alleviate, inhibit or eliminate said symptoms or progress. Desirable effects of treatment include preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, preventing metastasis, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis. In some disclosed embodiments, methods and compositions of the invention are used to delay development of a disease or disorder or to slow the progression of a disease or disorder.

[0071] Preferably, an effective amount, preferably a therapeutically effective amount of the protein or vector disclosed herein is administered. An 'effective amount' refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result. The effective amount may vary according to the drug or prodrug with which the protein or vector is co-administered.

[0072] A 'therapeutically effective amount' of a peptide of the invention may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the protein, to elicit a desired therapeutic result. A therapeutically effective amount encompasses an amount in which any toxic or detrimental effects of the protein are outweighed by the therapeutically beneficial effects. A therapeutically effective amount also encompasses an amount sufficient to confer benefit, e.g., clinical benefit.

[0073] Throughout the specification, terms such as 'comprises', 'comprised', 'comprising' and can have the meaning attributed to them in most patent jurisdictions, preferably in the jurisdiction in question; e.g. they can mean 'includes', 'included', 'including', etc. Terms such as 'consisting of' 'consisting essentially of' and 'consists essentially of' have the meaning ascribed to them in most patent jurisdictions, preferably in the jurisdiction in question; e.g., they may

imply the exclusion of all, most, or all but a negligible amount of other elements, or they may allow for elements not explicitly recited, but exclude elements that are found in the prior art or that affect a basic or novel characteristic of the invention.

[0074] The term 'about' as used herein when referring to a measurable value such as an amount, a temporal duration, and the like, is meant to encompass variations of $\pm 20\%$ or $\pm 10\%$, more preferably $\pm 5\%$, even more preferably $\pm 1\%$, and still more preferably $\pm 0.1\%$ from the specified value, as such variations are appropriate to perform the disclosed methods.

[0075] The invention will now be described in more detail by means of the following nonlimiting figures and examples.

BRIEF DESCRIPTION OF THE FIGURES

[0076]

Figure 1 shows an alignment between the amino acid sequences of human, mouse, pig and bovine CD31. The sequence defined by amino acids 593 to 600 of SEQ ID NO: 1 (human CD31) and the corresponding sequence in mouse, pig and bovine CD31 is indicated by a line above the sequence.

Figure 2 shows the effect of P8F (SEQ ID NO 5) and P8R1 (SEQ ID NO 6) peptides on T-cell activation. Jurkat T cells were preincubated with the fluo 3-AM calcium probe and stimulated by cross-linking the TCR with anti-CD3 antibodies and secondary antibody F(ab')₂ fragments. The figure shows the effect of 60 µg/ml of each peptide on calcium mobilization following TCR stimulation as follows: No peptide control (squares), P8F (SEQ ID NO 5) peptide (circles) and P8RI (SEQ ID NO 6) peptide (triangles).

Figure 3 shows that immobilised P8RI onto aminated solid surfaces inhibits leukocyte adherence and activation. Peripheral blood mononuclear cells (5×10^5 cells/ml) were transferred in P8RI-coated and control Immulon® wells and stimulated with 1 µg/ml LPS for 60'. Control wells were coated with acetic acid (with EDC/S-NHS) as irrelevant source of COOH groups (Control) or left uncoated (Bare). *Left*, after 3 washes with PBS, Control wells contained several adherent leukocytes at variance with P8RI wells in which leukocytes were virtually absent. *Right*, soluble L-selectin (sCD62L) and interleukin-1 β released from LPS-activated leukocytes (analyzed by cytometric beads, CBA®), were significantly reduced in P8RI-coated wells. * $p < 0.05$ vs Control. Scale bar=100 µm

Figures 4-6 show that immobilised P8RI prevents platelet activation. Thrombin generation was measured at 37°C by calibrated automated thrombogram in platelet rich plasma in the presence of Tissue Factor (0.5 pM) in aminated polystyrene wells. P8RI was covalently bound onto the wells. A scramble peptide was used as negative control *Fig 4*; Representative thrombograms; *Fig 5*: Velocity index of thrombin activity, calculated as Peak / Time to peak - LagTime, was significantly reduced in P8RI vs Bare and Control wells. *Fig 6*: The concentration

of soluble P-selectin (sCD62P), released by platelets, was significantly reduced in the supernatant of P8RI-coated wells. Data are from 8 independent experiments, * $p < 0.01$

Figure 7 shows the effect of the soluble peptides on VCAM-1 expression in D3 endothelial cells. The effect of P8RI on the expression of VCAM-1 was evaluated by quantitative FACS analysis on the immortalized human brain endothelial cell Line HCMEC/D3 submitted to overnight stimulation with TNF α (50ng/ml) and IFN γ (100ng/ml).

Figure 8 demonstrates a therapeutic effect of P8RI peptide in experimental autoimmune encephalitis, a mouse model of multiple sclerosis. A curative assay was performed in the EAE mouse model by starting the administration of P8RI at 2mg/kg/day subcutaneously after the onset of ascending paralysis (score ≥ 1), using prednisone as a pharmaceutical control. The figure shows ascending paralysis (clinical score) linked to active encephalitis in mice treated with vehicle (squares), prednisone at 2mg/kg/day (circles) or P8RI at 2mg/kg/day (triangles).

Figure 9 demonstrates a therapeutic effect of P8F peptide in a mouse model of acute thrombosis. Atherosclerotic mice were treated with P8F at 2.5, 5 or 10 mg/kg/day, with aspirin as a positive control and vehicle as a negative control. The number of sections showing an occlusive thrombus, out of 1000 sections/sample, was significantly lower in P8F-treated mice as compared to vehicle. No occlusive thrombus could be found in either the aspirin or the 5mg/ml group

Figure 10 shows a Cochran-Mantel-Haenszel test (Chi Square) analysis of the data presented in Figure 9. The width of the columns is proportional to the number of mice included in each group (Aspirin=5, "2.5"=7, "5"=11, "10"=7, Vehicle=8). $P < 0.05$ for all groups vs vehicle (Veh).

Brief description of the sequence listing

[0077]

SEQ ID NO: 1 corresponds to the sequence of human CD31.

SEQ ID NO: 2 corresponds to the sequence of murine CD31.

SEQ ID NO: 3 corresponds to the sequence of bovine CD31.

SEQ ID NO: 4 corresponds to the sequence of pig CD31.

SEQ ID NO: 5 corresponds to the P8F peptide. H-RVFLAPWK-OH

SEQ ID NO: 6 corresponds to the P8RI peptide. H-kwpalfvr-OH

SEQ ID NO: 7 corresponds to the human equivalent of the P8F peptide. H-RVILAPWK-OH

SEQ ID NO: 8 corresponds to the human equivalent of the P8RI peptide H-kwpalivr-OH

Examples***Example 1: Effect on T-cell activation:***

[0078] Jurkat T cells ($10 \times 10^6/\text{ml}$) were preincubated with the fluo 3-AM calcium probe and stimulated by cross-linking the TCR with anti-CD3 antibodies and secondary antibody F(ab')₂ fragments. Fig 2 shows continuous flow cytometry acquisition before and after addition of the stimulus \pm the peptides. As shown in the figure, the presence of 60 $\mu\text{g}/\text{ml}$ of P8RI (H-kwpalfvr-OH) and P8F (H-RVFLAPWK-OH) lead to a delayed and reduced intracellular calcium mobilization (as determined by the median fluorescence intensity (FMI) of the fluo-3AM probe, following TCR stimulation of Jurkat cells.

Example 2: Effect of the soluble peptide on platelet aggregation:

[0079] The microcapillaries of Vena8 Fluoro+™ biochip (400 μm width x 100 μm depth x 20 mm length, Cellix) were coated with 2.5 mg/ml insoluble horse type I collagen overnight at 4°C and then blocked with 0.1% HSA for 1 hour at room temperature. Human peripheral whole blood, anticoagulated with P-PACK (a direct thrombin inhibitor, non-chelating agent) was labeled with 5 μM DiOC6 for 10 minutes before perfusion with the Cellix pump at $1500 \text{ seconds}^{-1}$ through the coated capillaries. The interaction of platelets with the matrix was viewed in real time under a fluorescence microscope and was saved for off-line analysis. The surface covered by aggregated platelets was analyzed frame by frame (24 frames/s) over the first 5 minutes and expressed as coverage %. Soluble P8RI (H-kwpalfvr-OH) and P8F (H-RVFLAPWK-OH) were both shown to inhibit human platelet aggregation in a dose dependent manner.

Example 3: Effect of the immobilised peptide on platelet activation and thrombin generation.

[0080] Thrombin generation was measured at 37°C by calibrated automated thrombogram (Hemker et al. Thromb Haemost. 2000; 83(4): 589-91) in platelet rich plasma (1.5×10^8 platelets/ml) in the presence of Tissue Factor (0.5 pM) in aminated polystyrene wells (Covalink, Immulon® 2HB, Stago). SEQ ID NO 8 (P8RI, H-kwpalfvr-OH, MW 1016.26, TFA salt replaced by HCl salt, purity 100%, dissolved in water at 50 μM , pH 4-4.5) was pre-treated with EDC/S-NHS (10:1 molar ratio) and covalently bound onto the Immulon® wells. A scramble peptide was used as control peptide. Figs 4-6 show that thrombin activity, calculated as Peak / Time to peak - LagTime, was significantly reduced in P8RI vs Bare and Control wells, and that the

concentration of soluble P-selectin (sCD62P), released by platelets, was significantly reduced in the supernatant of P8RI-coated wells.

Example 4: Effect of the soluble peptide on endothelial cells:

[0081] The inventors evaluated the effect of P8RI on the expression of VCAM-1 by immunofluorescence on the immortalized human brain endothelial cell Line HCMEC/D3 submitted to overnight stimulation with TNFa (50ng/ml) and IFNg (100ng/ml). Although the increase in VCAM-1 expression was not impressive, the presence of the peptide prevented it (data not shown). Quantitative analysis by FACS of the same experimental conditions (Figure 7) showed that the reduction of VCAM-1 expression observed in the "peptide" wells was statistically significant (n=3 wells/condition). The effect was observed already at 6.25µg/ml and did not change with higher doses of the peptide (doses were up to 50µg/ml).

[0082] P8RI (H-kwpalfvr-OH (SEQ ID NO 6) MW 1016.26, TFA salt replaced by HCl salt, purity 100%, dissolved in water at 50 µM, pH 4-4.5) was pre-treated with EDC/S-NHS (10:1 molar ratio) and covalently bound onto aminated polystyrene wells (Covalink, Immunon® 2HB, Stago). Control wells were coated with acetic acid (with EDC/S-NHS) as irrelevant source of COOH groups. Wells were blocked with endothelial cell culture medium supplemented with 3% FCS prior to seeding primary Human umbilical endothelial cells (HUVECs, 5x10⁵ cells/ml). Three days later, P8RI-coated wells contained adherent and live cells (grey/translucent) while only dead cells/debris could be found in control wells (dark masses). This clearly demonstrated that immobilized P8RI favours endothelial cell attachment and growth onto solid supports.

Example 5: Curative protocol in a model of multiple sclerosis

[0083] To demonstrate effectiveness of the peptides in an in vivo therapeutic protocol, a curative assay was performed in the EAE mouse model (experimental autoimmune encephalitis, a mouse model of multiple sclerosis), with prednisone used as a reference drug. P8RI was administered at 2mg/kg/day, s.c. In the protocol used, the treatment started only once the clinical score was ≥1 (paralysis of the tail) and enrolled mice were followed up daily for 12 days. The reference drug (prednisone) was used at the same dosing schedule. As shown in Figure 8, the CD31 peptide was even more rapid and effective than prednisone in reducing the extent of the ascending paralysis (clinical score) linked to active encephalitis in treated mice.

Example 6: Curative protocol in a model of acute thrombosis

[0084] The P8F sequence was also used in vivo (subcutaneous administration of different doses: 2.5, 5 and 10mg/kg/d) in atherosclerotic mice (apoE KO, 35 weeks of age, male, chow

diet) subjected to the ligation of the left common carotid artery (a model of acute thrombosis). Due to advanced age, most of the mice had already developed an atherosclerotic lesion close to the site where the ligation was performed. Fluorescence photomicrography in cross sections stained with Evan's blue (fluorescent in the red channel) showed the occurrence of an occlusive thrombus over atherosclerotic lesions in untreated mice. The peptide was effective in preventing the appearance of an occlusive thrombus, at all the tested doses. The dose of 5mg/Kg/d showed the same extent of antithrombotic effect as the reference drug (aspirin, given subcutaneously at 150mg/kg/d). The number of sections showing an occlusive thrombus, out of 1000 sections/sample, was significantly lowered in P8F-treated mice as compared to vehicle (Figs 9 and 10). No occlusive thrombus could be found in either the aspirin or the 5mg/ml group.

Example :7 Comparison of P8F and P8RI with existing peptides

[0085] The solubility, activity and stability (resistance to freezing/thawing) of the P8F and P8RI peptides were compared to previously used peptides comprising the membrane-proximal sequences of CD31.

[0086] The peptides used for the comparison were:

P8F

P8RI

P23 Hum - amino acids 579-601 of the human CD31 sequence of SEQ ID NO: 1

P22 mouse - amino acids 569-590 of the mouse CD31 sequence of SEQ ID NO: 2

PepReg - amino acids 581-590 of the mouse CD31 sequence of SEQ ID NO: 2.

[0087] Activity was determined using the calcium mobilization test using Jurkat T cells, as described above. The results are shown in Table 1 below and are expressed as ED 50 (the minimal dose producing 50 percent of the maximum obtainable inhibition of intracellular calcium mobilization in Jurkat cells stimulated with antiCD3+Fab antibodies).

[0088] Stability was determined by subjecting the peptides to repeated freeze-thaw cycles and determining the number of cycles after which ED 50 was increased and/or biological activity abolished. The activity of the peptides was diminished or abolished after the number of freeze-thaw cycles indicated in Table 1

[0089] As can be seen from table 1 below, P8F and P8RI were superior to the previously used peptides in all respects tested. They showed increased solubility, lower ED 50 and equal or improved stability. In addition, whereas the previously used peptides required organic solvent,

P8F and P8RI were soluble in water, making them more suitable for medical applications.

Table I

	minimum purity (%)	minimal solvent	maximum soubility (mg/ml)	ED 50 (µM)	maximum freeze/thaw cycles
P8F	99	water	28,1	25	3
P8RI	100	water	30,5	12,5	5
P23 Hum	97	10% Acetonitrile	8,6	100	2
P22 mouse	96	10% Acetonitrile, 5% acetic acid	15,9	100	2
PepReg	98	10% Acetonitrile, 5% acetic acid	8,1	50	3

SEQUENCE LISTING

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		20						25					30		

Thr	Ile	Asn	Ser	Val	Asp	Met	Lys	Ser	Leu	Pro	Asp	Trp	Thr	Val	Gln
-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

35	40	45															
Asn	Gly	Lys	Asn	Leu	Thr	Leu	Gln	Cys	Phe	Ala	Asp	Val	Ser	Thr	Thr		
50						55					60						
Ser	His	Val	Lys	Pro	Gln	His	Gln	Met	Leu	Phe	Tyr	Lys	Asp	Asp	Val		
65					70					75					80		
Leu	Phe	Tyr	Asn	Ile	Ser	Ser	Met	Lys	Ser	Thr	Glu	Ser	Tyr	Phe	Ile		
				85					90					95			
Pro	Glu	Val	Arg	Ile	Tyr	Asp	Ser	Gly	Thr	Tyr	Lys	Cys	Thr	Val	Ile		
			100					105					110				
Val	Asn	Asn	Lys	Glu	Lys	Thr	Thr	Ala	Glu	Tyr	Gln	Leu	Leu	Val	Glu		
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Gln	Lys	Asp	Lys	Ala	Ile	Val	Ala	His	Asn	Arg	His	Gly	Asn	Lys	Ala		
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Lys	Val	Glu	Ser	Ser	Arg	Ile	Ser	Lys	Val	Ser	Ser	Ile	Val	Val	Asn		
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Ile	Thr	Glu	Leu	Phe	Ser	Lys	Pro	Glu	Leu	Glu	Ser	Ser	Phe	Thr	His		
				325					330					335			

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				645					650					655		
Met	Glu	Ala	Asn	Ser	His	Tyr	Gly	His	Asn	Asp	Asp	Val	Arg	Asn	His	
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Ala	Met	Lys	Pro	Ile	Asn	Asp	Asn	Lys	Glu	Pro	Leu	Asn	Ser	Asp	Val	
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Gln	Tyr	Thr	Glu	Val	Gln	Val	Ser	Ser	Ala	Glu	Ser	His	Lys	Asp	Leu	
	690					695					700					
Gly	Lys	Lys	Asp	Thr	Glu	Thr	Val	Tyr	Ser	Glu	Val	Arg	Lys	Ala	Val	
705					710					715					720	
Pro	Asp	Ala	Val	Glu	Ser	Arg	Tyr	Ser	Arg	Thr	Glu	Gly	Ser	Leu	Asp	
				725					730					735		
Gly	Thr															
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1				5					10					15		
Ala	Glu	Glu	Asn	Ser	Phe	Thr	Ile	Asn	Ser	Ile	His	Met	Glu	Ser	Leu	
			20					25					30			
Pro	Ser	Trp	Glu	Val	Met	Asn	Gly	Gln	Gln	Leu	Thr	Leu	Glu	Cys	Leu	
		35					40					45				
Val	Asp	Ile	Ser	Thr	Thr	Ser	Lys	Ser	Arg	Ser	Gln	His	Arg	Val	Leu	
	50					55					60					
Phe	Tyr	Lys	Asp	Asp	Ala	Met	Val	Tyr	Asn	Val	Thr	Ser	Arg	Glu	His	
65					70					75					80	
Thr	Glu	Ser	Tyr	Val	Ile	Pro	Gln	Ala	Arg	Val	Phe	His	Ser	Gly	Lys	
				85					90					95		
Tyr	Lys	Cys	Thr	Val	Met	Leu	Asn	Asn	Lys	Glu	Lys	Thr	Thr	Ile	Glu	
			100					105					110			
Tyr	Glu	Val	Lys	Val	His	Gly	Val	Ser	Lys	Pro	Lys	Val	Thr	Leu	Asp	
		115					120					125				
Lys	Lys	Glu	Val	Thr	Glu	Gly	Gly	Val	Val	Thr	Val	Asn	Cys	Ser	Leu	
	130					135					140					

Gln Glu Glu Lys Pro Pro Ile Phe Phe Lys Ile Glu Lys Leu Glu Val
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Gly Thr Lys Phe Val Lys Arg Arg Ile Asp Lys Thr Ser Asn Glu Asn
165 170 175

Phe Val Leu Met Glu Phe Pro Ile Glu Ala Gln Asp His Val Leu Val
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Phe Arg Cys Gln Ala Gly Ile Leu Ser Gly Phe Lys Leu Gln Glu Ser
195 200 205

Glu Pro Ile Arg Ser Glu Tyr Val Thr Val Gln Glu Ser Phe Ser Thr
210 215 220

Pro Lys Phe Glu Ile Lys Pro Pro Gly Met Ile Ile Glu Gly Asp Gln
225 230 235 240

Leu His Ile Arg Cys Ile Val Gln Val Thr His Leu Val Gln Glu Phe
245 250 255

Thr Glu Ile Ile Ile Gln Lys Asp Lys Ala Ile Val Ala Thr Ser Lys
260 265 270

Gln Ser Ser Glu Ala Val Tyr Ser Val Met Ala Met Val Glu Tyr Ser
275 280 285

Gly His Tyr Thr Cys Lys Val Glu Ser Asn Arg Ile Ser Lys Ala Ser
290 295 300

Ser Ile Met Val Asn Ile Thr Glu Leu Phe Pro Lys Pro Lys Leu Glu
305 310 315 320

Phe Ser Ser Ser Arg Leu Asp Gln Gly Glu Leu Leu Asp Leu Ser Cys
325 330 335

Ser Val Ser Gly Thr Pro Val Ala Asn Phe Thr Ile Gln Lys Glu Glu
340 345 350

Thr Val Leu Ser Gln Tyr Gln Asn Phe Ser Lys Ile Ala Glu Glu Ser
355 360 365

Asp Ser Gly Glu Tyr Ser Cys Thr Ala Gly Ile Gly Lys Val Val Lys
370 375 380

Arg Ser Gly Leu Val Pro Ile Gln Val Cys Glu Met Leu Ser Lys Pro
385 390 395 400

Ser Ile Phe His Asp Ala Lys Ser Glu Ile Ile Lys Gly His Ala Ile
405 410 415

Gly Ile Ser Cys Gln Ser Glu Asn Gly Thr Ala Pro Ile Thr Tyr His
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Leu Met Lys Ala Lys Ser Asp Phe Gln Thr Leu Glu Val Thr Ser Asn
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Asp Pro Ala Thr Phe Thr Asp Lys Pro Thr Arg Asp Met Glu Tyr Gln
450                               455                     460

Cys Arg Ala Asp Asn Cys His Ser His Pro Ala Val Phe Ser Glu Ile
465                               470                     475                     480

Leu Arg Val Arg Val Ile Ala Pro Val Asp Glu Val Val Ile Ser Ile
                               485                     490                     495

Leu Ser Ser Asn Glu Val Gln Ser Gly Ser Glu Met Val Leu Arg Cys
                               500                     505                     510

Ser Val Lys Glu Gly Thr Ser Pro Ile Thr Phe Gln Phe Tyr Lys Glu
                               515                     520                     525

Lys Glu Asp Arg Pro Phe His Gln Ala Val Val Asn Asp Thr Gln Ala
530                               535                     540

Phe Trp His Asn Lys Gln Ala Ser Lys Lys Gln Glu Gly Gln Tyr Tyr
545                               550                     555                     560

Cys Thr Ala Ser Asn Arg Ala Ser Ser Met Arg Thr Ser Pro Arg Ser
                               565                     570                     575

Ser Thr Leu Ala Val Arg Val Phe Leu Ala Pro Trp Lys Lys Gly Leu
                               580                     585                     590

Ile Ala Val Val Val Ile Gly Val Val Ile Ala Thr Leu Ile Val Ala
595                               600                     605

Ala Lys Cys Tyr Phe Leu Arg Lys Ala Lys Ala Lys Gln Lys Pro Val
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Glu Met Ser Arg Pro Ala Ala Pro Leu Leu Asn Ser Asn Ser Glu Lys
625                               630                     635                     640

Ile Ser Glu Pro Ser Val Glu Ala Asn Ser His Tyr Gly Tyr Asp Asp
645                               650                     655

Val Ser Gly Asn Asp Ala Val Lys Pro Ile Asn Gln Asn Lys Asp Pro
660                               665                     670

Gln Asn Met Asp Val Glu Tyr Thr Glu Val Glu Val Ser Ser Leu Glu
675                               680                     685

Pro His Gln Ala Leu Gly Thr Arg Ala Thr Glu Thr Val Tyr Ser Glu
690                               695                     700

Ile Arg Lys Val Asp Pro Asn Leu Met Glu Asn Arg Tyr Ser Arg Thr
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Glu Gly Ser Leu Asn Gly Thr
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<213> Bos taurus

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Leu  Thr  Leu  Leu  Leu  Cys  Ser  Ser  Leu  Lys  Gly  Gln  Glu  Asn  Ser  Phe
      20      25      30

Thr  Ile  Asn  Ser  Ile  His  Met  Gln  Ile  Leu  Pro  His  Ser  Thr  Val  Gln
      35      40      45

Asn  Gly  Glu  Asn  Leu  Thr  Leu  Gln  Cys  Leu  Val  Asp  Val  Ser  Thr  Thr
      50      55      60

Ser  Arg  Val  Lys  Pro  Leu  His  Gln  Val  Leu  Phe  Tyr  Lys  Asp  Asp  Val
65      70      75      80

Leu  Leu  His  Asn  Val  Ser  Ser  Arg  Arg  Asn  Thr  Glu  Ser  Tyr  Leu  Ile
      85      90      95

Pro  His  Val  Arg  Val  Cys  Asp  Ser  Gly  Arg  Tyr  Lys  Cys  Asn  Val  Ile
      100     105     110

Leu  Asn  Asn  Lys  Glu  Lys  Thr  Thr  Pro  Glu  Tyr  Glu  Val  Trp  Val  Lys
      115     120     125

Gly  Val  Ser  Asp  Pro  Arg  Val  Thr  Leu  Asp  Lys  Lys  Glu  Val  Ile  Glu
      130     135     140

Gly  Gly  Val  Val  Val  Val  Asn  Cys  Ser  Val  Pro  Glu  Glu  Lys  Ala  Pro
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Val  His  Phe  Thr  Ile  Glu  Lys  Phe  Glu  Leu  Asn  Ile  Arg  Gly  Ala  Lys
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Lys  Lys  Arg  Glu  Lys  Thr  Ser  Gln  Asn  Gln  Asn  Phe  Val  Thr  Leu  Glu
      180     185     190

Phe  Thr  Val  Glu  Glu  Gln  Asp  Arg  Thr  Ile  Arg  Phe  Gln  Cys  Gln  Ala
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Lys  Ile  Phe  Ser  Gly  Ser  Asn  Val  Glu  Ser  Ser  Arg  Pro  Ile  Gln  Ser
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Asp  Leu  Val  Thr  Val  Arg  Glu  Ser  Phe  Ser  Asn  Pro  Lys  Phe  His  Ile
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Ile  Pro  Glu  Gly  Lys  Val  Met  Glu  Gly  Asp  Asp  Leu  Gln  Val  Lys  Cys
      245     250     255

Thr  Val  Gln  Val  Thr  His  Gln  Ala  Gln  Ser  Phe  Pro  Glu  Ile  Ile  Ile
      260     265     270

Gln  Lys  Asp  Arg  Glu  Ile  Val  Ala  His  Asn  Ser  Leu  Ser  Ser  Glu  Ala
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Lys Val Glu Ala Ser Arg Ile Ser Lys Val Ser Ser Val Val Val Asn
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Val Thr Glu Leu Phe Ser Lys Pro Lys Leu Glu Ser Ser Ala Thr His
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Val Tyr Leu Pro Leu Glu Lys Gly Leu Ile Ala Val Val Val Ile Gly
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Glu Ala Asp Arg His Tyr Gly Tyr Asn Glu Asp Val Gly Asn His Ala
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Tyr Thr Glu Val Glu Val Thr Ser Pro Glu Pro His Gln Gly Leu Gly
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Lys Val Glu Ala Ser Arg Ile Ser Lys Val Ser Ser Ile Met Val Asn
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Leu Asp Gln Gly Glu Ser Leu Arg Leu Trp Cys Ser Ile Pro Gly Ala
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Pro Pro Glu Ala Asn Phe Thr Ile Gln Lys Gly Gly Met Met Met Leu
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Gln Asp Gln Asn Leu Thr Lys Val Ala Ser Glu Arg Asp Ser Gly Thr
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Tyr Thr Cys Val Ala Gly Ile Gly Lys Val Val Lys Arg Ser Asn Glu
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Val Gln Ile Ala Val Cys Glu Met Leu Ser Lys Pro Ser Ile Phe His
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Lys	Trp	Pro	Ala	Leu	Ile	Val	Arg
1				5			

REFERENCES CITED IN THE DESCRIPTION

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Patentkrav

1. Isoleret peptid bestående af:
 - a) aminosyresekvensen af aminosyrerne 582 til 589 ifølge SEQ ID NO: 2;
 - 5 b) et fragment af mindst 6 efterfølgende aminosyrer fra (a);
 - c) aminosyresekvensen svarende til (a) eller (b) i et andet CD31 fra et pattedyr; eller
 - d) en aminosyresekvens svarende til (a), (b) eller (c), hvor peptidets fulde aminosyresekvens er inverteret, hvor begrebet inverteret betyder en inversion af aminosyrekæden fra den C-terminale ende til den N-terminale ende,
 - 10 hvor peptidet er opløseligt i vand og, hvor peptidet har den biologiske aktivitet med udøvelse af en dosisafhængig hæmning af T-celleproliferation *in vitro*.
2. Peptid ifølge krav 1(c), hvor det andet CD31 fra et pattedyr er et humant, porcint eller bovint CD31.
- 15 3. Peptid ifølge krav 1 eller 2, hvor én eller flere aminosyrer er i D-enantiomer-formen.
4. Peptid ifølge et hvilket som helst af kravene 1 til 3, hvor peptidet har sekvensen:
 - (i) H-RVFLAPWK-OH (SEQ ID NO: 5);
 - 20 (ii) H-kwpalfvr-OH (SEQ ID NO: 6);
 - (iii) H-RVILAPWK-OH (SEQ ID NO: 7) eller
 - (iv) H-kwpalivr-OH (SEQ ID NO: 8).
5. Peptid ifølge et hvilket som helst af kravene 1 til 4, hvor peptidet er bundet til en fast
25 bærer.
6. Peptid ifølge krav 5, hvor den faste bærer er en intravaskulær protese.
7. Peptid ifølge krav 6, hvor den intravaskulære protese er en stent.
- 30 8. Farmaceutisk sammensætning, der omfatter peptidet ifølge et hvilket som helst af kravene 1 til 7.
9. Peptid ifølge et hvilket som helst af kravene 1 til 7 til anvendelse i behandling af en

trombotisk eller en inflammatorisk lidelse.

10. Peptid til anvendelse ifølge krav 9, hvor lidelsen er:

- en trombotisk lidelse, der er udvalgt fra gruppen bestående af aterotrombose, aterosklerose, akut koronært syndrom, iskæmisk apopleksi, periferisk arteriesygdom og abdominal aortaaneurisme; eller

- en inflammatorisk lidelse, der er udvalgt fra gruppen bestående af rheumatoid arthritis, multipel sklerose, inflammatorisk tarmsygdom, systemisk lupus erythematosus, Graves' sygdom, diabetes mellitus, psoriasis, atopisk dermatitis, cerebral amyloid angiopati og vasculitis.

11. Peptid til anvendelse ifølge krav 9 eller 10, hvor lidelsen er kendetegnet ved et øget antal af CD31⁺-T-lymfocytter fra perifert blod og/eller forhøjede plasmaniveauer af spaltet opløseligt CD31.

12. Peptid til anvendelse ifølge et hvilket som helst af kravene 9 til 11, hvor peptidet er bundet til en fast bærer, såsom en intravaskulær protese.

13. Peptid til anvendelse ifølge et hvilket som helst af kravene 9 til 11, hvor peptidet skal indgives subkutant.

DRAWINGS

HUMAN	MQPRWAQGATMWLGVLTLTLLCSSLEGOENSFTINSVDMKSLPDWTVQNGKNLTLOCFAD	60
MOUSE	----- MLLALGLTLVLYASLOAEENSFTINSIHMESLPSWEVMNGOQLTLECLVD	50
BOVIN	MQLRWTQRGMMWLGALLTLLCSSLKGOENSFTINSIHMQILPHSTVQNGENLTLOCLVD	60
PIG	MRLRWTQGGNMWLGVLLTLQLCSSLEGOENSFTINSIHMEMLPGQEVHNGENLTLOCIVD	60
HUMAN	VSTTSHVKPQHOMLFYKDDVLFYNISSMKSTESYFIPQARVYDSGTYKCTVIVNNKEKTT	120
MOUSE	ISTTSKSRSQHRVLFYKDDAMVYNVTSREHTESYVIPQARVFHSGKYKCTVMLNNKEKTT	110
BOVIN	VSTTSRVKPLHQVLFYKDDVLLHNVSRRNTESYLI PHVRVCDSGRYKCNVILNNKEKTT	120
PIG	VSTTSSVKPPQHQLVLFYKDDVLFHNVSSTKNTESYFISEARVYNSGRYKCTVILNNKEKTT	120
HUMAN	AEYQLLVEGVSPRVTLDKKEAIOGGIVRVNCSVPPEEKAPIHFTTIEKLELNEKVMVKLKRE	180
MOUSE	IEYEVKVHGVSKPKVTLDKKEVTEGGVTVNCSLQEEKPPIFFKIEKLEVGTKFVKRRRID	170
BOVIN	PEYEVWVKGVSDPRVTLDKKEVIEGGVVVNCVPEEKAPVHFTIEKFELNIRGAKKKRE	180
PIG	AEYKVVVEGVSNPRVTLDKKEVIEGGVVKVTCVPEEKPPVHFTIEKFELNVRDVQQRRE	180
HUMAN	KNSRDQNFVILEFPVEEQDRVLSFRRCQARIISGIHMQTSESTKSELVTVTSEFSTPKFHI	240
MOUSE	KTS-NENFVLMFPIEADHVLVFRCCQAGILSGFKLOESEPIRSEYVTVQSEFSTPKFEI	229
BOVIN	KTSQNQNFVTLTEFTVEEQDRTIRFQCQAKIFSGSNVESRRPIQSDLVTVRESFSNPKFHI	240
PIG	KTANNQNSVTLEFTVEEQDRVILFSCQANVIFGTRVEISDSVRSDLVTVRESFSNPKFHI	240
HUMAN	SPTGMIMEGAQLHIKCTIQVTHLAQEFPEII IQDKAIVAHRHGNKAVYSVMAMVEHSG	300
MOUSE	KPPGMIIEGDQLHIRCIVQVTHLVQEFTEII IQDKAIVATSKQSSSEAVYSVMAMVEYSG	289
BOVIN	IPEGKVMEGDDLQVKCTVQVTHQAQSFPEII IQKDREIVAHSNLSSEAVYSVMATTEHNG	300
PIG	SPKGVIIIEGDQLLIKCTIQVTHQAQSFPEII IQKDKEIVAHSRNGSEAVYSVMATVEHNS	300
HUMAN	NYTCKVESRIRISKVSSIVVNIITELFSKPELESSFTHLDQGERLNLSCSIPGAPP-ANFTI	359
MOUSE	HYTCKVESNRISKASSIMVNIITELFPKPKLEFSSSRLLDQGEILLDSCSVSGTPV-ANFTI	348
BOVIN	NYTCKVEASRISKVSSVVVNVVTELEFSKPKLESSATHLDQGEDLNLCSIPGAPP-ANFTI	359
PIG	NYTCKVEASRISKVSSIMVNIITELFSRPKLKSSATRLDQGESLRLWCSIPGAPPEANFTI	360
HUMAN	QKEDTIVSQIQDETAKIASKSDSGTYICTAGIDKVVKKSNVTQIVVCEMLSQPRISYDAQF	419
MOUSE	QKEETVLSQYQNFESKIAEESDSGEYSCTAGIGKVVKRSGLVPIQVCEMLSKPSIFHDAKS	408
BOVIN	QKGGMTVSQTQNFETKRVSEWDSGLYTCVAGVGRVFKRSNTVQITVCEMLSKPSIFHDSRS	419
PIG	QKGGMMLQDQNLTKVASERDSGTYTCTVAGIGKVVKRSNEVQIAVCEMLSKPSIFHDSGS	420

FIG.1
Beginning

HUMAN	EVIKGQTIEVRCEISISGTLPISYQLLKTSKVLNSTKNSNDPAVFKDNPTEDEVYQC	479
MOUSE	EIIKGHAIGISCQSENGTAPITYHLMKAKSDFQTLLEVTSTNDPATFTDKPTRDMEYQCR	468
BOVIN	EVIKGQTIEVSCQSVNGTAPIFYQLSNTSKPVANQSVGSNKPALFRVKPTTKDVEYCCS	479
PIG	EVIKGQTIEVSCQISINGTSPISYQLLKGSDDLASQNVSSNEPAVFKDNPTKDVEYQCI	480
HUMAN	NCHSHAKMLSEVLRVKVIAPVDEVQISILSSKVVESGEDIVLQCAVNEGSGPITYKIFYR	539
MOUSE	NCHSHPAVFSEILRVRIAPVDEVVISILSSNEVQSGSEMVLRCVKEGTSPIITFQIFYK	528
BOVIN	NCHSHSKMFSVLRVKVIAPVDEAQL-VVLKGEVEPGEPVIFYCSVNEGSPITYKIFYK	538
PIG	NCHSHAGMPFSKVLRVKVIAPVEEVKLSILLSEEVESGQAIVLQCSVKEGSGPITYKIFYK	540
HUMAN	KEGKPFYQMTSNATQAFWTKQASKEQEGEYYCTAFNRANHASSVPRSKILLTVRVILAP	599
MOUSE	KEDRPFHQAVVNDTQAFWHNKQASKKQEGQYYCTASNRASSMRTPSRSSLAVRVFLAP	588
BOVIN	KESKPFYQDTINATQIMWHKTTASKKEYEGQYYCTASNRAVLSKHVIQSNLTITVRVYL-PL	597
PIG	KENKPFHQVTLNDTQAIWHKPKASKDQEGQYYCLASNRAVTPSKNFIQSNILAVRVYLAP	600
HUMAN	KKGLIAVVIGVIGVIAALLIIAAKCYFLRKAKAKQKMPVEMSRPAVPLLNSNNEK-MSDPNME	658
MOUSE	KKGLIAVVIGVIGVIAATLIIVAACYFLRKAKAKQKPVEMSRPAAPLNSNSEK-ISEPSVE	647
BOVIN	EKGLIAVVIGVIVTLVLGAKCYFLKKAKAKQKMPVEMSRPAVPLLNSNNEKTLSDAGTE	657
PIG	KKGLIAVVIAVIAVLLLGARFYFLKKS KAKQKMPVEMCRPAAPLNSNNEKTLSDPNT	660
HUMAN	ANSHYGHNDVVRNHAMKPINDNKEPLNSDVQYTEVQVSSAESHKDLGKKD--TETVYSEV	716
MOUSE	ANSHYGYDDVSGNDVAVKPINQNKDPQNMDVEYTEVEVSSLEPHQALGTRA--TETVYSEI	705
BOVIN	ADRHYGYNEDVGNHAMKPLNENKEPLTLDVEYTEVEVTSPEPHQGLGTEGTETVYSEI	717
PIG	ANRHYGYNEDVGNHAMKPLNENKEPLTLDVEYTEVEVTSPEPHRGLGTEGT--TETVYSEI	718
HUMAN	RKAVPDAVESRYSRTEGSLDGT	738
MOUSE	RKVDPNLMENRYSRTEGSLNGT	727
BOVIN	RKADPDFVENRYSRTEGSLDGS	739
PIG	RKADPDLVENRYSRTEGSLDGT	740

FIG.1 End

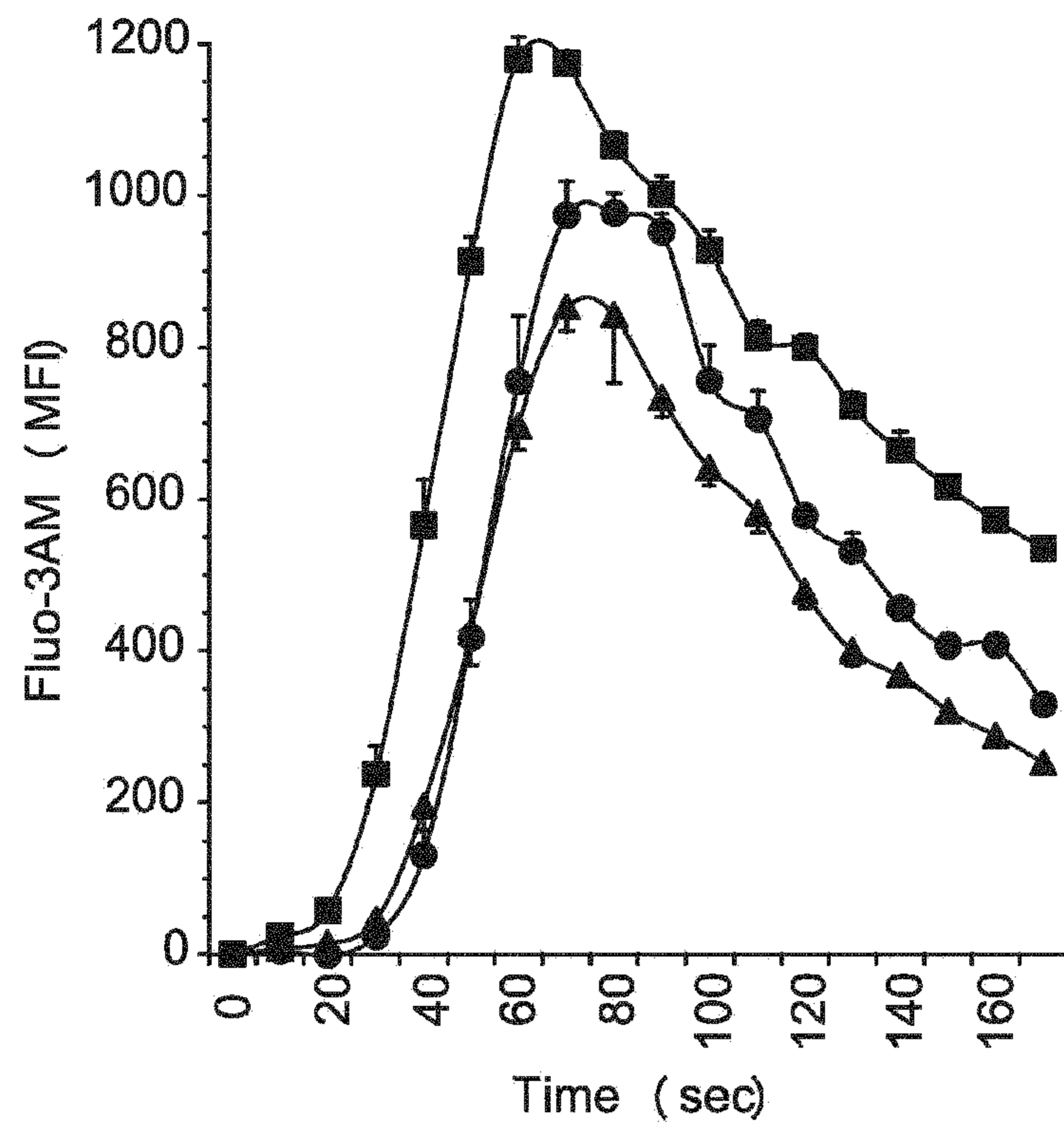
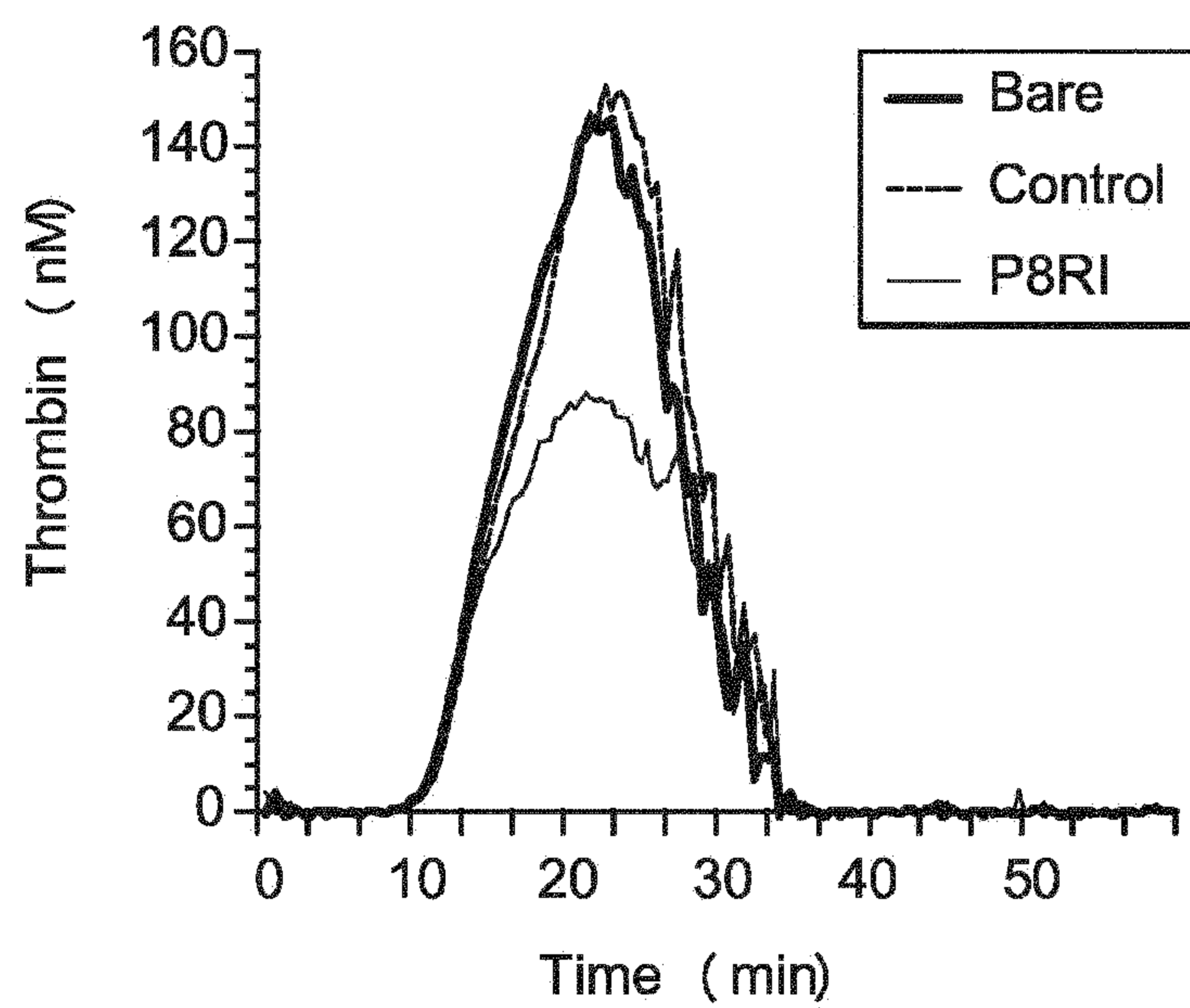
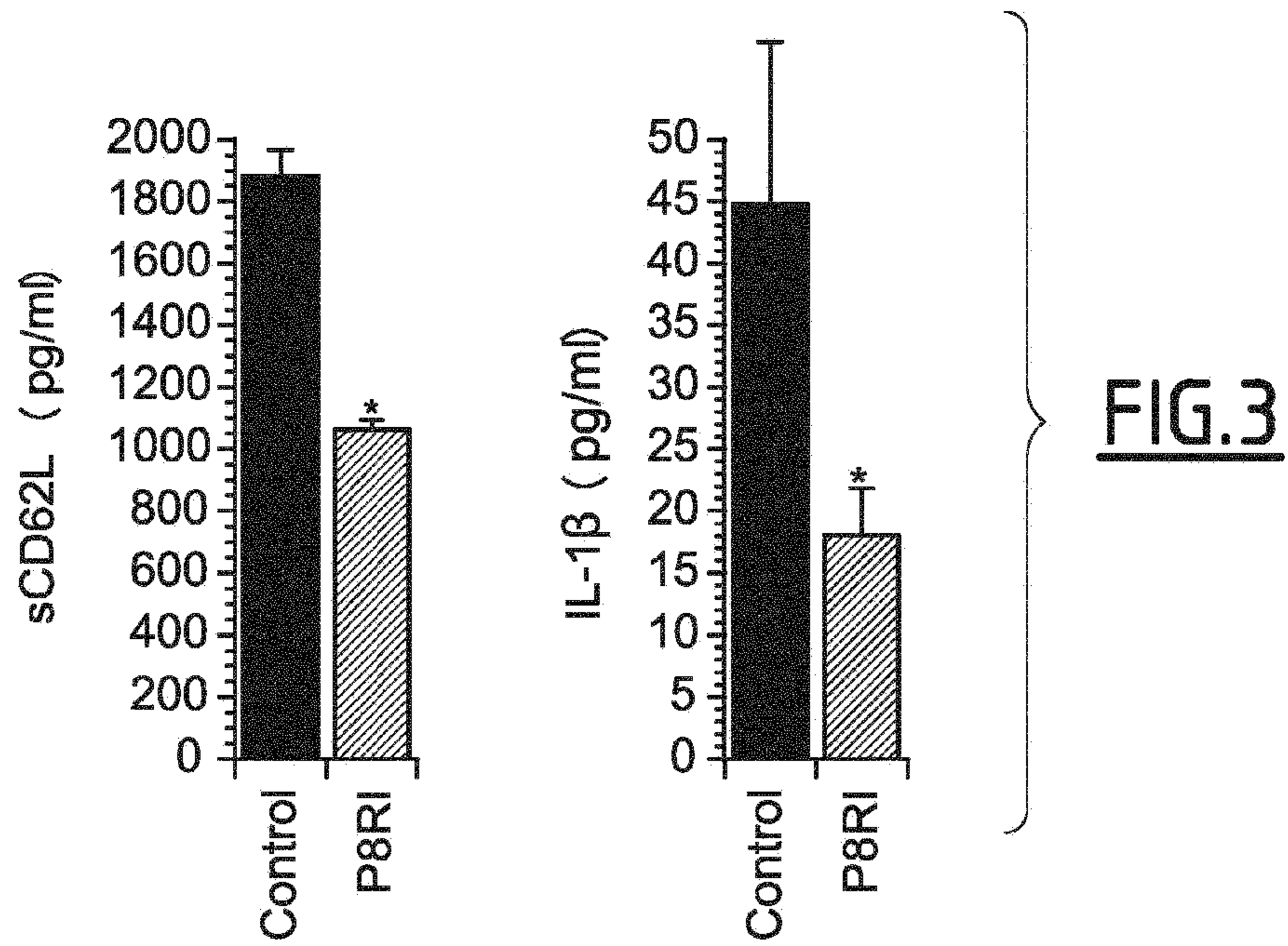


FIG.2

**FIG.4**

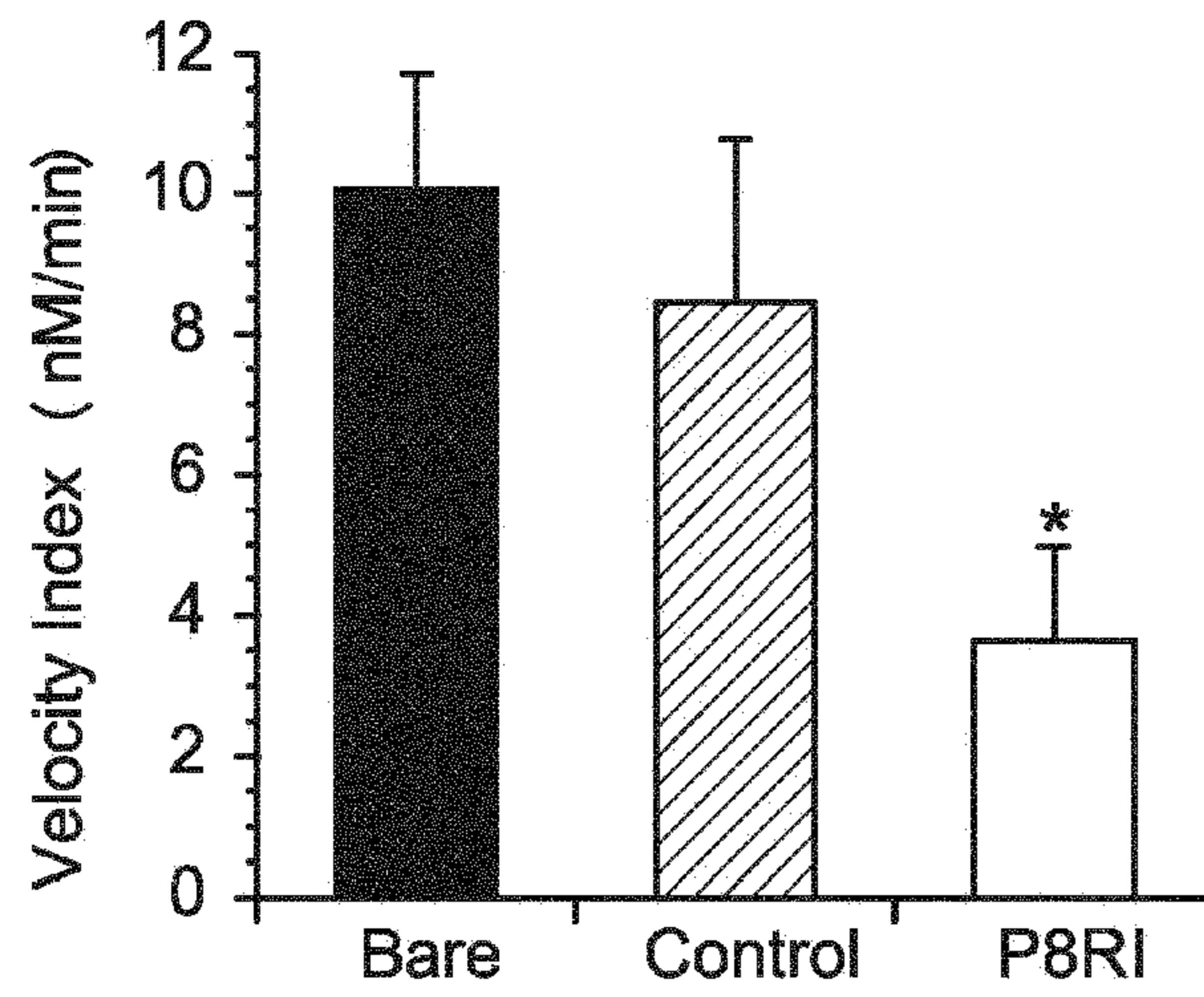


FIG.5

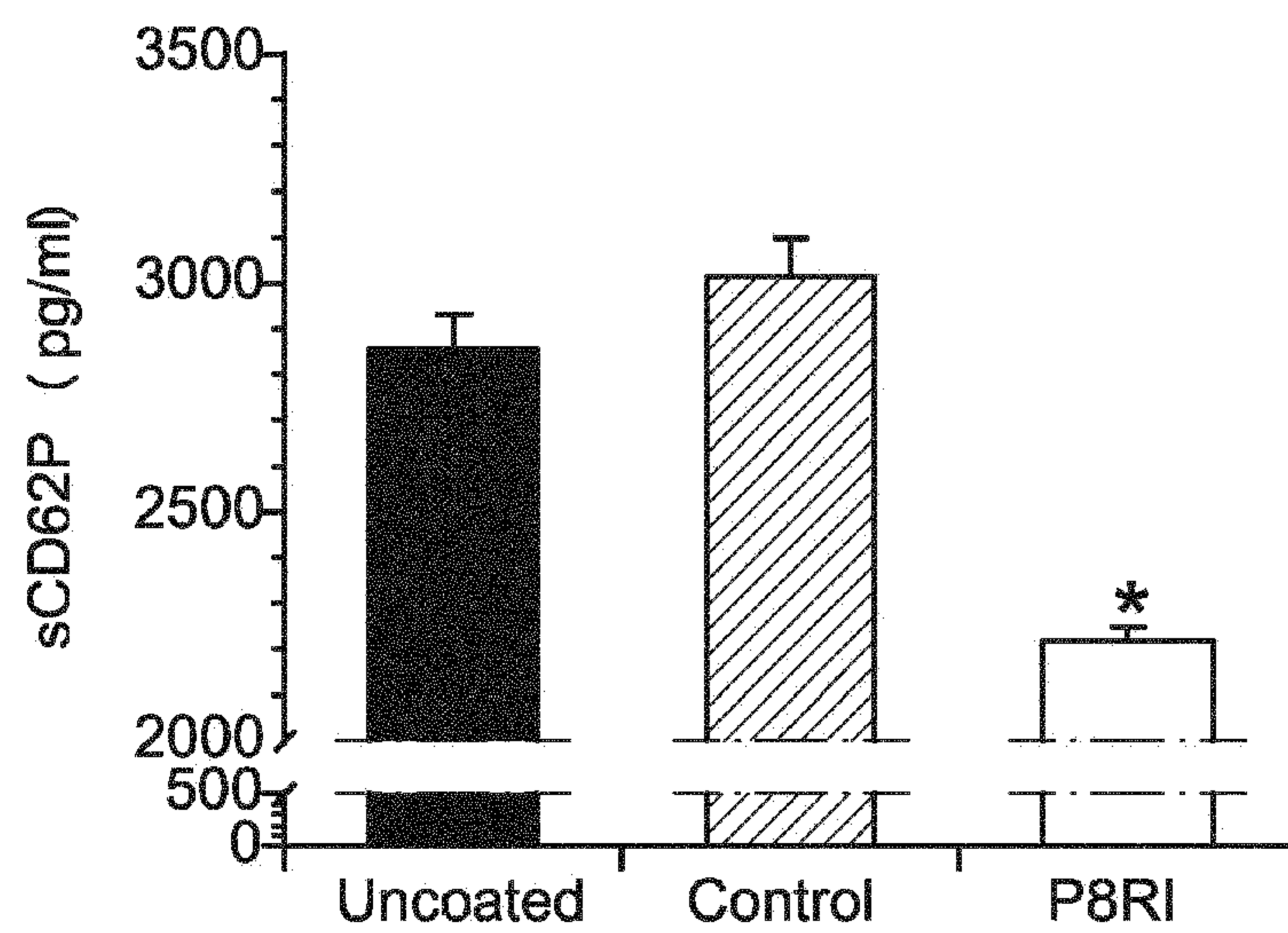
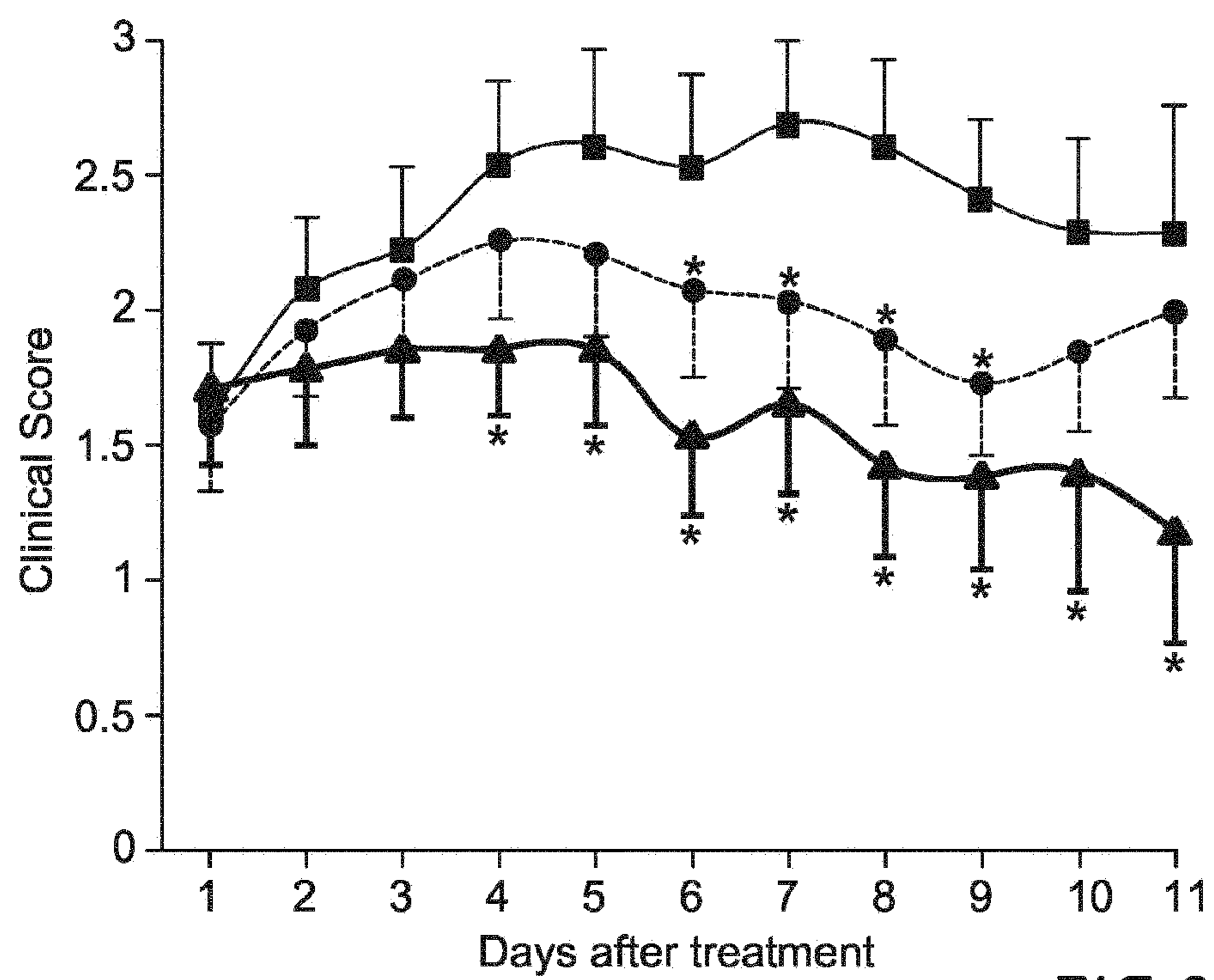
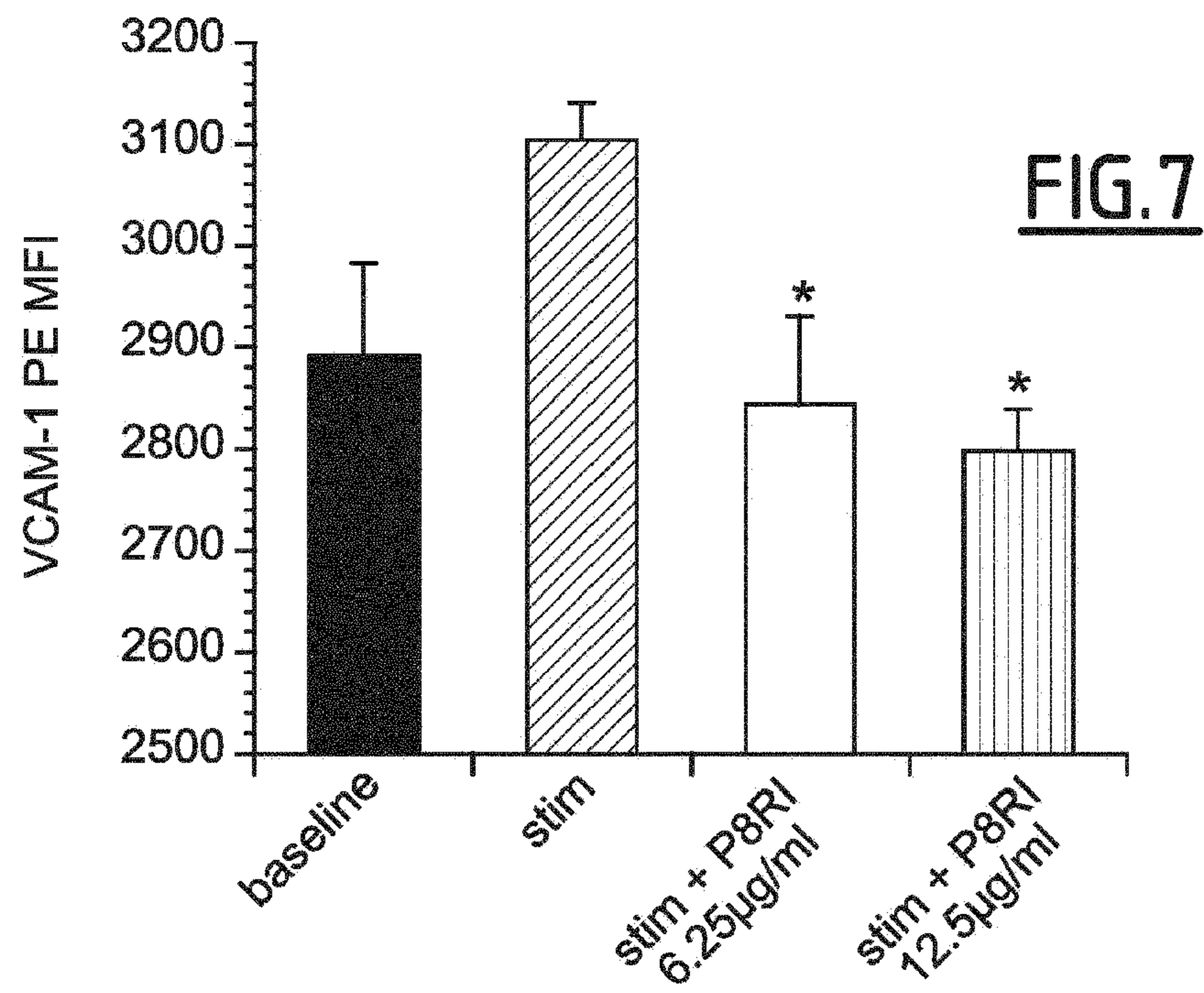


FIG.6



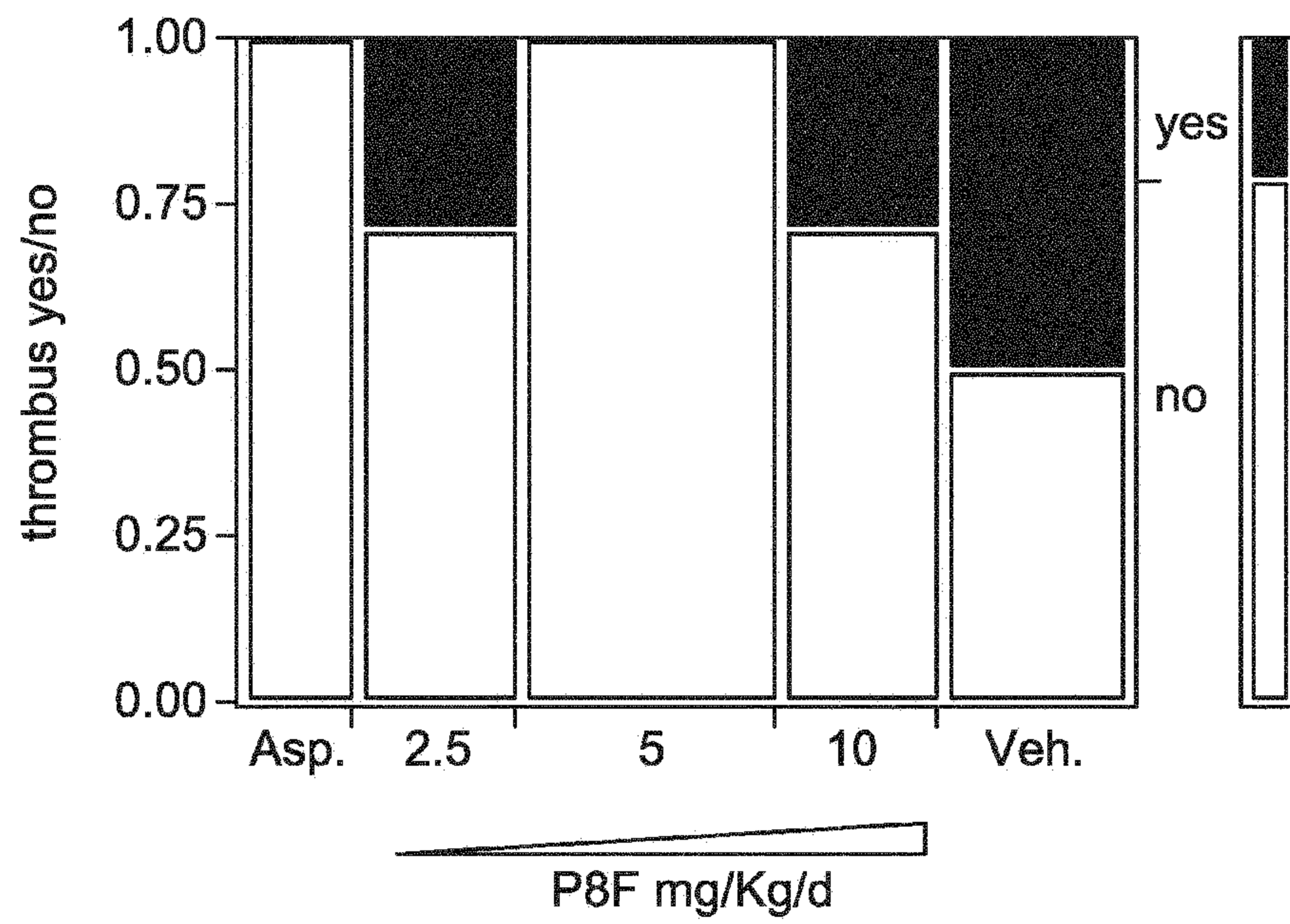
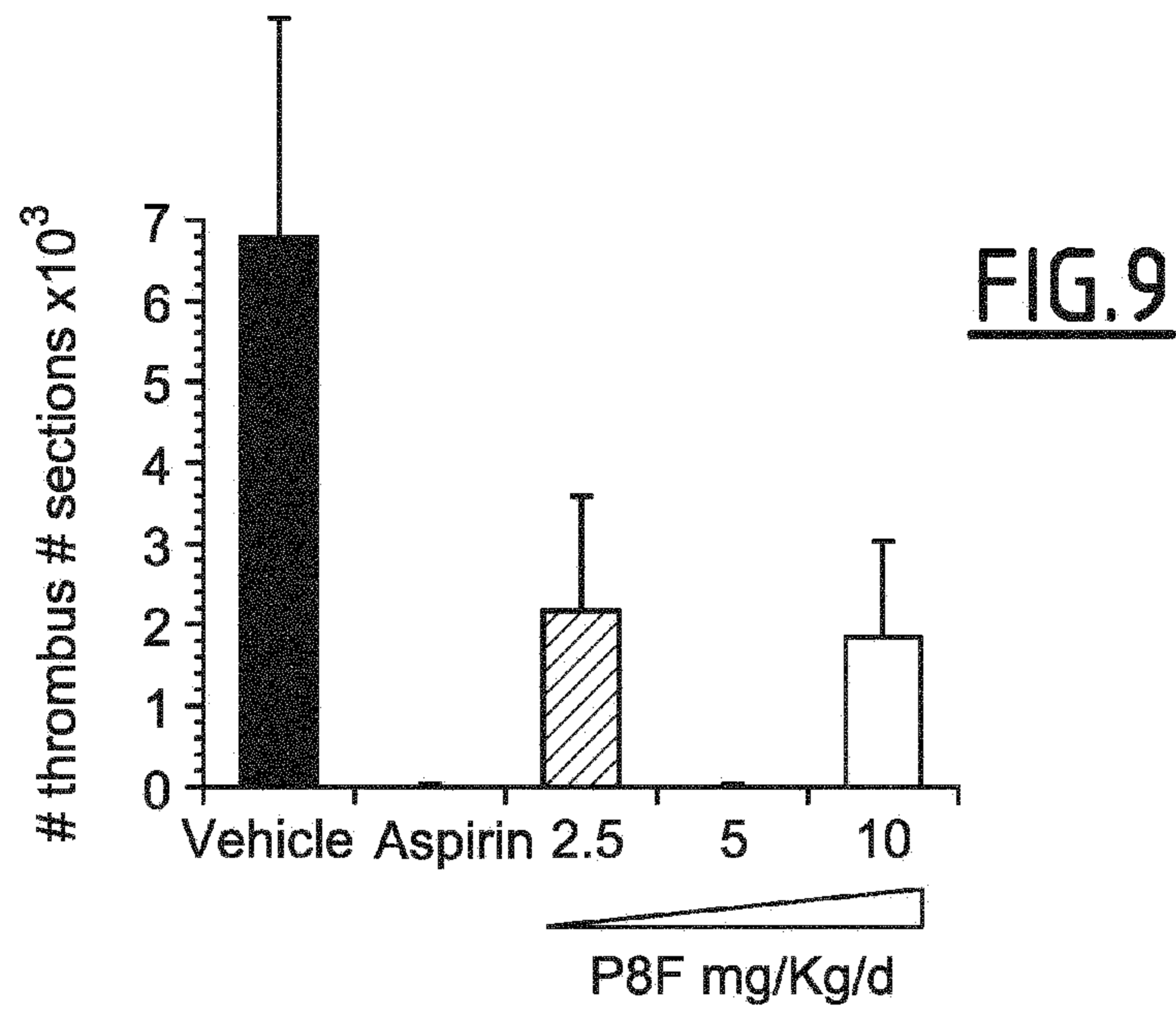


FIG.10