METHODS OF USE OF $\beta$1-INTEGRIN INHIBITORS

The present invention provides methods that use agents capable of one or more of the following: inhibiting leukocyte mediated destruction of tissues, especially after injuries to tissue due to heat or cold (i.e., burns or frostbite), light, electricity, chemicals, or other agents that cause tissue injury and destruction; inhibiting leukocyte mediated destruction of tissues after myocardial infarction, CNS ischemic injury (e.g., stroke), angioplasty, surgical incisions, injury-mediated trauma, and transplant reperfusion; inhibiting angiogenesis; inhibiting cancer cell metastasis, motility, and/or migration within tissue; restoring and/or potentiating the induction of programmed cell death in cancerous tissues or restoring normal cellular phenotype to cancerous tissue; inhibiting osteoclast adhesion and bone resorption; and/or inhibiting stem cell adhesion to bone marrow.
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METHODS OF USE OF β1-INTEGRIN INHIBITORS

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Statement of Related Applications
The present invention claims priority to U.S. Provisional Application Serial No. 60/125,634, filed March 22, 1999, and U.S. Provisional Application Serial No. 60/167,538, filed November 24, 1999, both of which are incorporated herein by reference.

10 Background of the Invention

Integrins are a family of cell surface proteins that mediate the interactions of cells with their environment. They are heterodimeric proteins that include two membrane glycoproteins, a larger α subunit and a smaller β subunit. The β2 subunits are largely involved in cell-cell interactions, while β1 subunits are associated with mediating cell adhesion to extracellular matrix constituents, including extracellular matrix (ECM) macromolecules reactive with β1 subunit containing integrin molecules, such as fibronectin, laminin, tenascin, and Type IV collagen. See, for example, International Publication No. WO 99/37669.

15 As cell surface proteins, integrins regulate a variety of cellular interactions including cellular adhesion to extracellular matrices, cell-cell association, and cellular motility/migration within tissues/organs. They also are considered important mediators of a variety of pathological conditions, including acute inflammation, cancer, and osteoporosis, and serve to localize and/or promote development/differentiation of cells within specific tissue and/or organ compartments, e.g., stem cell localization and differentiation.

20 Inflammation

Inflammation is required to orchestrate the recovery of tissue integrity, immune competence, and homeostasis following severe injury. Acute inflammation
represents the first step of this biological process; however, if the inflammatory response is excessive in initial magnitude or duration, this otherwise beneficial process can contribute to the further deterioration rather than the restoration of tissue/organ homeostasis.

Acute inflammation induced by CNS ischemic injury and thermal/cold injury represent two clinical conditions wherein this two-edged sword of inflammation operates. In each of these situations, similar events occur subsequent to the reperfusion of the injured tissue(s). These events determine whether the injury extends outward from the initial focus of the injury to include additional tissue, or recover, thus containing the injury to the initial site and limiting any additional loss of functional tissue. In addition to burns and stroke, a wide variety of clinical situations share this inflammation-mediated progression of injury size and scope, including myocardial infarction, angioplasty, surgical incisions, injury-mediated trauma and transplant reperfusion.

Much of the acute inflammatory response within tissues is mediated by leukocytes. That is, leukocytes have been implicated in the pathogenesis and progression of microvascular injury and extravascular tissue damage. To reach the site of injury, leukocytes must adhere to the vessel/capillary wall and then migrate to the injured tissue. Thus, leukocyte-mediated injury is dependent in part on polymorphonuclear neutrophil adherence to endothelial cell surface and leukocyte aggregation in extracellular tissue.

Leukocyte adherence to endothelial cells and to extracellular matrix components is mediated by multiple adhesion receptor systems. In the first stages of inflammation, leukocyte rolling into inflammatory sites is mediated by the selectin family of adhesion receptors. Additional cellular recognition receptors, the integrins, then mediate leukocyte binding to the endothelium. Once leukocytes migrate across the endothelial cell membrane their adherence to the extracellular matrix is directed by many factors, including integrins.

There is increasing evidence that integrins represent one important set of mediators of the pathological events associated with acute inflammation.
particular, β1-integrins have recently been determined to play a critical role in the adhesion and migration of leukocytes (PMNs) into tissues at the site of injury, as the expression of β1-integrins is elevated 25-fold on extravasated PMNs over the level of β1-integrins expressed on circulating PMNs. Similar elevation of β1-integrin expression is observed in other biological and/or pathological conditions.

Cancer

β1-integrins also play important roles in cancer cell differentiation, tumor metastasis, angiogenesis, and regulation of tumor cell apoptosis-modulators of cancerous tissue growth and spread. As increased expression of β1-integrins has been demonstrated to correlate with prognosis and metastatic potential (i.e., higher levels of β1-integrins correlate with higher metastatic potential), β1-integrins appear to coordinately regulate critical parameters within cancerous tissue. The levels of β1-integrin are often increased on cancerous cells/tissues, thus altering the interactions of cancer cells with their surroundings and inducing changes in the cancer cell’s phenotype.

Osteoporosis

Integrins mediate the interactions of osteoclasts with their environment and thus the actions of osteoclasts. Of the several integrins expressed on the surfaces of osteoclasts, β1-integrins are one of those expressed at the highest levels. Recent studies have determined β1-integrin activity to correlate with the adhesive and bone-resorptive capacity of osteoclasts.

Stem Cell Localization

Bone marrow transplant (either allogeneic or autologous) represents an increasingly utilized approach to managing a variety of hematopoietic and cancerous disease states. Current methodologies require the direct harvesting of donor bone marrow, a painful and invasive medical procedure. This approach limits the availability of donor stem cell materials. Enhanced methods of obtaining
hematopoietic stem cells, for example, by cellular phoresis, would increase the utilization of bone marrow transplant as a strategy for the treatment of cancer, AIDS, and genetic disease (gene therapy).

There are numerous agents that have been described that modulate integrin activity. Only two classes of agents, however, have been described that interact both selectively in the extracellular space with the β subunit of β1-integrins and possess the capacity to inhibit the activity of one or more integrin heterodimer complexes. These two classes include antibodies and the peptides disclosed in International Publication No. WO 99/37669, the latter of which are the only ones known to interact preferentially for activated β1-integrins. Inhibitory antibodies are known to possess the capability of binding and blocking the activity of integrin β1 subunits; however, these tend to be large and not very accessible to pathological sites.

Thus, there is a need for additional agents that modulate (e.g., inhibit) integrin activity, particularly those that have better access to pathological sites than antibodies. For example, there is still a need in the art for other agents that can inhibit inflammatory leukocyte mediated destruction of tissues, especially during ischemia reperfusion injury resulting from CNS ischemic injury (e.g., stroke), myocardial infarction, angioplasty, surgical incisions, injury-related trauma, and transplant reperfusion. There is still a need for agents that can inhibit inflammatory leukocyte mediated destruction of tissues subsequent to injuries induced by exposure to heat, cold, light, electricity, chemicals, or other agents that cause tissue injury and destruction.

In addition, there is still a need for agents that can: inhibit angiogenesis; inhibit cancer cell metastasis, motility, and/or migration within tissues; and restore/potentiate the induction of programmed cell death in cancerous tissues. Also, there is still a need for agents that can inhibit osteoclast adhesion and bone resorption. Furthermore, there is still a need for agents that can promote the peripheralization of hematopoietic stem cells for the purposes of harvesting said
stem cells and using them for various transplant procedures and methodologies including autologous stem cell transplantation to restore hematopoietic potential subsequent to cyto-reductive therapies (cancer chemo- and radio-therapeutics and the like), or for the purposes of generating specific immune cells for use in the production of cell based immunogens for cancer, viruses, pathogens and the like.

**Summary of the Invention**

The present invention provides methods that involve the use of β1-integrin inhibitors. In one embodiment, a method of inhibiting inflammatory leukocyte mediated destruction of tissue in a patient is provided. The method includes administering to the patient a composition comprising a β1-integrin inhibitor. As used herein, a β1-integrin inhibitor is an agent that inhibits β1 subunit containing integrin irrespective of the associated α subunit.

In this method, the inflammatory leukocyte mediated destruction of tissue can occur as a result of CNS ischemic injury, myocardial infarction, angioplasty, surgical incisions, injury-related trauma, transplant reperfusion, or a combination thereof. Alternatively, the inflammatory leukocyte mediated destruction of tissue can occur as a result of exposure to heat, cold, light, electricity, chemicals, or a combination thereof. The injury that results from any of these conditions is referred to herein as a “burn-type” injury.

In a preferred embodiment of the present invention, there is provided a method of treating a stroke patient. The method includes administering to the patient a composition that includes a β1-integrin inhibitor in an amount effective to reduce infarct size, reduce neurological deficit, or both. Preferably, such administration occurs within about 3 hours after the stroke occurs.

In another preferred embodiment, there is provided a method of treating a patient having a burn-type injury. The method includes administering a composition that includes a β1-integrin inhibitor in an amount effective and over a period of time effective to reduce leukocyte-mediated tissue destruction. The period of time is preferably at least 1 hour. Also, preferably the administration is topical.
In certain embodiments, the composition is administered periodically over a predetermined period of time.

In yet another embodiment, there is provided a method of treating a burn patient that involves maintaining a composition that includes an effective amount of a $\beta_1$-integrin inhibitor on a burn-type injury for a period of time effective to reduce leukocyte-mediated tissue destruction and achieve a desired degree of healing.

The present invention also provides a method of treating a cancer patient. The method involves administering to the patient a composition that includes a $\beta_1$-integrin inhibitor in an amount effective to inhibit one or more of angiogenesis, cancer cell metastasis, cancer cell motility, or cancer cell migration. Alternatively, the method involves administering a $\beta_1$-integrin inhibitor in an amount effective to induce programmed cell death in cancerous tissue or restore normal cellular phenotype to cancerous tissue.

A method of treating a patient for osteoporosis is also provided. The method involves administering to the patient a composition that includes a $\beta_1$-integrin inhibitor in an amount effective to inhibit osteoclast adhesion and bone resorption.

A method of peripheralizing stem cells is also provided. This method involves administering to a patient a composition comprising a $\beta_1$-integrin inhibitor.

Yet another embodiment of the present invention is a composition that includes a $\beta_1$-integrin inhibitor and a pharmaceutically acceptable carrier. As used herein, "a" means one or more, such that combinations of inhibitors can be used in the compositions and methods of the invention.

Preferably, the $\beta_1$-integrin inhibitor is a peptide that has a C-terminal LipAr motif. More preferably, the $\beta_1$-integrin inhibitor is a peptide comprising an amino acid sequence selected from the group consisting of WQPRARIY (SEQ ID NO:___), QPPRAAIY (SEQ ID NO:___), WQPPAARIY (SEQ ID NO:___), AQPPRAIRY (SEQ ID NO:___), WAPPRARY (SEQ ID NO:___), WQPPDADIY (SEQ ID NO:___), ARITGYIY (SEQ ID NO:___), RARITGYIY (SEQ ID NO:___), PRQAWRPIY (SEQ ID NO:___), RPAPQRWIY (SEQ ID NO:___).
ARITGYIY (SEQ ID NO: __), PRARIY (SEQ ID NO: __), RARIY (SEQ ID NO: __), ARIY (SEQ ID NO: __), and RIIY.

**Brief Description of the Figures**

The invention can be better understood with reference to the following detailed description together with the appended illustrative drawings in which like elements are numbered the same:

Figure 1 is a bar chart of the Doppler blood flow in marginal zones of burn study. This shows blood flow in burn zones at baseline, 24, 48, and 72 hours.

Control \(-80.56 \pm 3.58, 29.97 \pm 2.94, 42.67 \pm 4.38, 52.17 \pm 5.06\); 24 hour treatment \(-78.33 \pm 2.64, 42.25 \pm 3.40, 46.63 \pm 2.69, 62.62 \pm 4.69\); 48 hour treatment \(-70.69 \pm 2.07, 56.23 \pm 2.55, 65.70 \pm 2.95, 75.19 \pm 3.30\). Laser Doppler perfusion measurement in zones of stasis presented as mean ± SEM for controls (solid bar), 24-hour treatment group (broken bar) and 48-hour treatment group (white bar) (*p < 0.05 versus Baseline by t-test; +p < 0.05 versus Control by t-test). Control animals had significant decreases in perfusion in the zones of stasis at all post-burn time points. Animals in the 24-hour treatment group had higher perfusion than controls at 24-hour post burn. Animals in the 48-hour treatment group had higher blood flow in the zones of stasis at 24, 48, and 72 hours versus controls.

Figure 2 is a bar chart of marginal zone Doppler blood flow.

Figure 3 is graph of data showing rabbit weight changes for seven days.

Figure 4 is graph of data showing daily temperature changes for seven days.

Figure 5 is graph of data showing body temperatures for seven days.

Figure 6 is graph of data showing daily white blood cell count for seven days.

Figure 7 is graph of data showing daily hematocrit for seven days.

Figure 8 is a photograph of the zones of stasis in a group of control animals.

Figure 9 is a photograph of the zones of stasis in a group of animals treated for 24 hours with Trp-9-Tyr.

Figure 10 is a photograph of the zones of stasis in a group of animals treated
for 48 hours with Trp-9-Tyr.

Figure 11 is table of data showing abscess formation at day 7 in burn study.

Figure 12 is table of data showing abscess size at day 7 in burn study.

Figure 13 is a bar chart of infarction size of MCAO (stroke) study.

Figure 14 is a bar chart of neurological index results of MCAO (stroke) study.

Figure 15 is a graph of the inhibition of Ramos Adhesion to tumor necrosis factor alpha-stimulated human vascular endothelial cells; V = WQPPRARIY (SEQ ID NO: ____); sV = scrambled (inactive) version of V which has an amino acid sequence of RPQIPWARY (SEQ ID NO: ____); sCS-1 = scrambled version of CS-1; TNF = tumor necrosis factor alpha.

Figure 16 is graph of the inhibition of Ramos Adhesion to tumor necrosis factor alpha-stimulated human vascular endothelial cells; a4 = inhibitory antibody against the alpha-4 integrin molecule; B1 = B1 inhibitory antibody; n. mouse = normal mouse serum.

Figure 17 is a graph of the time course of WQPPRARIY (SEQ ID NO: ____)
in human plasma at 37°C with varying initial concentrations.

Figure 18 is a graph of average (SD) concentrations of WQPPRARIY (SEQ ID NO: ____)
in rat blood versus time during two-phase crossover IV infusion study. The rats were given a low dose treatment (○) first at 5 mg/kg/min for 15 minutes, and immediately after this, the animals received a high dose infusion (□) at 10 mg/kg/min for 15 minutes.

**Detailed Description of Preferred Embodiments of the Invention**

The present invention provides methods that use agents capable of one or more of the following: inhibiting leukocyte mediated destruction of tissues, especially after injuries to tissue due to heat or cold (i.e., burns or frostbite), light, electricity, chemicals, or other agents that cause tissue injury and destruction; inhibiting leukocyte mediated destruction of tissues after myocardial infarction, CNS ischemic injury (e.g., stroke), angioplasty, surgical incisions, injury-mediated
trauma, and transplant reperfusion; inhibiting angiogenesis; inhibiting cancer cell metastasis, motility, and/or migration within tissues; restoring and/or potentiating the induction of programmed cell death in cancerous tissues or restoring normal cellular phenotype to cancerous tissue; inhibiting osteoclast adhesion and bone resorption; and/or inhibiting stem cell adhesion to bone marrow.

The agents useful in the methods of the present invention are those that interact selectively with activated β1-integrin molecules (i.e., in the conformational state that permits/promotes interaction with ligand, which in this case are components of the extracellular matrix, such as fibronectin, laminin, collagen and the like, and/or cell surface molecules) relative to resting (i.e., unactivated) β1-integrin molecules. They are preferably smaller than antibodies to β1-integrins. Generally, monomeric IgG molecules (antibodies) are approximately 150 kDa while the active agents used herein are no greater than about 15 kDa, preferably no greater than about 5 kDa, and more preferably no greater than about 1000 daltons (1 kDa).

Furthermore, agents useful in the methods of the present invention do not induce an immuno-suppressed state. Significantly, selectivity for activated β1-integrin molecules may allow for systemic administration and the use of higher doses. Also, such agents are advantageous because they are believed to induce fewer side effects. Examples of such agents are described in International Publication No. WO 99/37669. These agents, as well as others (which may or may not be peptides) that can be developed based on structure-activity relationships defined by these agents, are useful in the methods of the present invention.

The methods of the present invention include treatments for inflammation (e.g., such as that resulting from burns), cancer, and osteoporosis, as well as methods of harvesting hematopoietic stem cells.

**Inflammation**

Integrins represent an important set of mediators of acute inflammation, with β1-integrins playing an important role in the adhesion and migration of leukocytes into tissues at the site of injury. Moreover, the expression of β1-integrins is elevated
25-fold on extravasated leukocytes over the level of β1-integrins expressed on circulating leukocytes. As such, inhibition of β1-integrin activity would likely provide an approach to mitigating the adverse effects of the acute inflammatory response, as it would block the migration and/or inflammatory responses elicited by leukocytes within tissues. Moreover, as integrins possess limited biological redundancy, are late effectors of critical biological processes, and can be blocked with limited side effects agents which block integrin activity are likely to possess limited side effects. Thus, agents that modulate β1-integrin activity are believed to provide superior therapeutic regulation of acute inflammation processes.

The agents of the present invention are believed to provide superior access to sites of inflammation. They also provide the structural basis/rationale for the development of small molecules (peptides, peptidomimetics, and/or small organic molecules) possessing enhanced anti-β1-integrin inhibitory activity.

A wide variety of clinical situations that share an inflammation-mediated progression of injury size and scope include surgical incisions, injury-mediated trauma, myocardial infarction, angioplasty, CNS ischemic injury (e.g., stroke), transplant reperfusion, heat, cold, light, electricity, chemicals, etc., can also be positively affected by the agents of the present invention. Other clinical situations can include injuries initiated by heat or cold (i.e., burns or frostbite), light, electricity, chemicals, or other agents that cause tissue injury and destruction. Thus, the present invention provides a method of inhibiting inflammatory leukocyte mediated destruction of tissue in a patient by administering to the patient an effective amount of a β1-integrin inhibitor.

In a preferred embodiment, the present invention provides a method that utilizes one or more agents capable of ameliorating leukocyte-mediated tissue destruction without suppressing leukocyte activity against foreign agents such as bacterial or viral agents. Such method results from the discovery that inhibition of WBC adherence with Trp-9-Tyr reduces the microvascular damage and progression of tissue necrosis following thermal injury. The synthetic fibronectin peptide (Trp-9-Tyr) has the amino acid sequence WQPPRARIY (SEQ ID NO:____).
Specifically, the present invention provides a method of treating a patient having a burn-type injury by administering a β1-integrin inhibitor in an amount effective and over a period of time effective to reduce leukocyte-mediated tissue destruction. The order of preference for the period of time is at least 1 hour, at least 4 hours, at least 8 hours, at least 12 hours, at least 24 hour, at least 36 hours, and at least 48 hours. Preferably, the mode of administration is topical and the administration is periodic.

Cancer

Inhibition of β1-integrin activity has been demonstrated to induce phenotypic restoration in cancer cells in vitro. Furthermore, inactivating antibodies against β1-integrins have been demonstrated to block tumor cell mediated angiogenesis, a process involved in tumor growth and expansion. Also, inactivating antibodies against β1-integrins block the interaction of β1-integrins with the extracellular matrix, which is rich in β1-integrin binding molecules, such as fibronectin, laminin, collagen, and tenascin that surround cancers, with β1-integrins, thereby restoring the induction of programmed cell death (apoptosis) in the cancerous cells and blocking cellular migration within tissues.

Thus, agents that modulate β1-integrin activity can serve as powerful regulators of cancer cell biology. Thus, small molecule inhibitors (peptides, peptidomimetics, and/or small molecules) of β1-integrins, such as the agents of the present invention, are believed to provide superior access to tumor sites and permit the therapeutic regulation of cancer cell phenotype. They also provide the structural basis/rationale for the development of small molecules (peptides, peptidomimetics, and/or small organic molecules) possessing enhanced anti-β1-integrin inhibitory activity. Induction/restoration of a normal cellular phenotype in tumor cells would provide for the development of superior cancer therapeutic regimens.

Cancers that can be treated using the methods of the present invention include, but are not limited to, breast cancer, melanoma, mesothelioma, and myeloma. Such treatment methods can involve, for example, the inhibition of
tumor cell metastasis, the inhibition of tumor cell motility, the inhibition of tumor cell migration, the enhancement of tumor cell apoptosis, the induction of targeted paralysis and reversion of cancerous cell phenotype, and/or the inhibition of tumor mediated angiogenesis.

Thus, the present invention provides a method of treating a cancer patient by administering a β1-integrin inhibitor in an amount effective to inhibit one or more of angiogenesis, cancer cell metastasis, cancer cell motility, or cancer cell migration.

10 Osteoporosis

Improved methods of modulating osteoclast function are necessary to provide enhanced treatment methodologies for osteoporosis and other bone-resorptive disease processes. Agents that modulate β1-integrin activity, such as those of the present invention, are believed to act as powerful regulators of osteoclast function, thus facilitating the therapeutic regulation of bone-resorptive disease processes. Such agents are believed to provide superior access to the sites where bone turnover is occurring. They also provide structural basis/rationale for the development of small molecules (peptides, peptidomimetics, and/or small organic molecules) possessing enhanced anti-β1-integrin inhibitory activity. Thus, the present invention provides a method of treating a patient for osteoporosis by administering a β1-integrin inhibitor in an amount effective to inhibit osteoclast adhesion and bone resorption.

Stem Cell Localization

Hematopoetic stem cells (CD34 cells) express high levels of β1-integrins. These molecules mediate their adherence to bone marrow cavity (stroma). Agents that that modulate β1-integrin activity (e.g., by blocking this interaction), such as the agents of the present invention, are believed to induce the peripheralization (movement into the circulation) of hematopoetic stem cells. This allows stem cells to be harvested using standard cellular phoresis methodologies, for example, thus
providing a readily available supply of donor hematopoietic stem cells. Thus, such agents are believed to permit the development of enhanced hematopoietic stem cell harvesting techniques. It is further believed that this would increase the availability of donor materials, thus increasing the number and variety of procedures for which bone marrow transplantation could be employed.

Thus, methods of the present invention include the use of agents that enhance the peripheralization of stem cells through their interaction with activated forms of β1-integrins on stem cells. Such methods can be used in conjunction with known methods for harvesting cells from the peripheral circulation system.

Optionally, cytokines, such as G-CSF, GM-CSF and/or Flt 3 ligand, can be used that enhance stem cell peripheralization. Stem cells obtained according to the present invention can be used to treat a variety of conditions, including, but not limited to, cancer, AIDS, and genetic disorders through gene therapy.

Inhibitory Agents and Compositions

Suitable agents for use in the methods of the present invention include peptides with a C-terminal amino acid residue having a side chain that includes an aromatic group (“-Ar-”) and an amino acid residue with a lipophilic alkyl side chain group (“-Lip-“) as the penultimate C-terminal residue, as exemplified in International Publication WO 99/37669. This C-terminal dipeptide sequence is referred to herein as a “LipAr motif.” These peptides with a C-terminal LipAr motif and are typically capable of inhibiting β1-integrin subunit dependent cell adhesion and, in particular, of inhibiting α4β1-integrin dependent cell adhesion, and typically α2β1, α3β1, and/or α5β1 integrin dependent cell adhesion.

Examples of suitable amino acid residues having an aromatic group include tyrosine (“Tyr”), phenylalanine (“Phe”), histidine (“His”), and tryptophan (“Trp”). The penultimate C-terminal “Lip” residue is an amino acid residue that includes a lipophilic alkyl side chain group. The α-carboxyl group of the C-terminal amino acid residue of the present peptides is typically in the form of a carboxylic acid (-CO₂H). In a preferred embodiment of the invention, the “Lip” and “Ar” residues
are L-amino acid residues. The following standard single letter code abbreviations are used to designate the amino acid residues in the peptides: A - alanine, C - cysteine, D - aspartate, E - glutamate, F - phenylalanine, G - glycine, H - histidine, I - isoleucine, K - lysine, L - leucine, M - methionine, N - asparagine, P - proline, Q - glutamine, R - arginine, S - serine, T - threonine, V - valine, W - tryptophan, Y - tyrosine.

Examples of amino acid residues that have a lipophilic alkyl side chain group include leucine (“Leu”), isoleucine (“Ile”), and valine (“Val”). Typically, the lipophilic alkyl side chain group has a SCDC (cyclohexane-water side chain distribution coefficient calculated as -RT ln K_D and expressed in kcal/mol) of at least about 3.0 and, preferably, at least about 4.0. For the purposes of this application, SCDC is defined according to Radzicka et al., Biochemistry, 27, 1664 (1988). Where the SCDC of a particular alkyl side chain group is not known, the SCDC value may be determined by measurement of the distribution coefficient between wet cyclohexane and water or by a comparison of a compound containing the same alkyl side chain group with other similar compounds using a hydrophobicity scale derived from HPLC retention according to the method of Parker et al., Biochemistry, 25, 5425 (1986). Despite its similarity in some respects to lipophilic alkyl side chain groups such as leucine, isoleucine, and valine, insertion of a methionine residue at the penultimate position (i.e., an “MY” C-terminal motif) resulted in an inactive analog.

Suitable peptides with a C-terminal Lip-Ar motif for use in the methods of the present invention include and WQPPRARIY (SEQ ID NO:__). The alanine knockout analogs (i.e., an analog of a peptide in which a single residue has been substituted by an alanine residue) of the latter peptide that preserve the C-terminal LipAr motif (i.e., retain the C-terminal Ile-Tyr dipeptide sequence) can also be used in the methods of the present invention. Two of the alanine knockout analogs of WQPPRARIY (SEQ ID NO:__) have an alanine residue substituted for one of the arginine residues in the “PRARI” motif (Pro-Arg-Ala-Arg-Ile (SEQ ID NO:__)). These alanine knockout analogs have the amino acid sequences WQPPRAAIY
(SEQ ID NO:__) and WQPPAARIY (SEQ ID NO:__). Two of the other alanine knockout analogs, AQPPRARIY (SEQ ID NO:__), WAPPRAIY (SEQ ID NO:__), also differ from WQPPRAIY (SEQ ID NO:__) by a non-conservative amino acid substitution (Ala for Trp and Ala for Gln respectively).

Peptides that differ from WQPPRAIY (SEQ ID NO:__) by a non-conservative amino acid substitution but retain the C-terminal LipAr motif can be capable of modulating β1 integrin subunit dependent cell adhesion even if the overall physical properties of the peptide differ substantially from WQPPRAIY (SEQ ID NO:__). For example, an analog of this peptide in which the two arginine residues have been replaced by aspartic acid residues is suitable for use in the methods of the present invention, as is WQPPDADIY (SEQ ID NO:__), which has an overall net charge of -2 (in contrast to the +2 net charge of WQPPRAIY (SEQ ID NO:__).

For certain preferred embodiments of the methods of the present invention, other suitable peptides include those that contain no more than 10 amino acid residues and have a sequence that does not correspond substantially to the amino acid sequence of WQPPRAIY (SEQ ID NO:__). As used herein, the sequence of a particular peptide does not correspond substantially to a reference amino acid sequence, if the particular peptide sequence has less than about 80% identity and preferably less than about 50% homology with the reference sequence. As used herein, the term “% homology” refers to the percentage of amino acid residues of a peptide which are either identical to that of an original peptide sequence or differ from the original peptide sequence solely as a result of a conservative amino acid substitution. For example, the peptide PAIFDRSCGS (SEQ ID NO:__) has 40% identity and 80% homology with respect to the peptide sequence PKVMERTCDS (SEQ ID NO:__).

Even peptides with less than 50% homology with the corresponding C-terminal portion of WQPPRAIY (SEQ ID NO:__) exhibit the capability of inhibiting β1 integrin subunit dependent adhesion, and thus are suitable for use in the methods of the present invention. Examples of such peptides include
ARITYII (SEQ ID NO:__), RARITYII (SEQ ID NO:__), PRQAWRPIY (SEQ ID NO:__), and RPAPQRWIY (SEQ ID NO:__). One group of particularly suitable peptides of the invention are those which include a C-terminal "IIY" motif, i.e., the sequence of the three C-terminal most amino acid residues is Ile-Ile-Tyr. One such peptide contains 9 amino acid residues and has the sequence ARITYII (SEQ ID NO:__). Another group of particularly advantageous peptides of the invention include the C-terminal IY motif and contain no more than ten and, preferably, no more than six amino acid residues. In addition to the dipeptide Ile-Tyr, suitable examples of this group include PRARYI (SEQ ID NO:__), RARIY (SEQ ID NO:__), ARY (SEQ ID NO:__), and RIY.

For the purposes of this invention, conservative amino acid substitutions are defined to result from exchange of amino acids residues from within one of the following classes of residues: Class I: Ala, Gly, Ser, Thr, and Pro (representing small aliphatic side chains and hydroxyl group side chains); Class II: Cys, Ser, Thr and Tyr (representing side chains including an -OH or -SH group); Class III: Glu, Asp, Asn and Gln (carboxyl group containing side chains); Class IV: His, Arg and Lys (representing basic side chains); Class V: Ile, Val, Leu, Phe and Met (representing hydrophobic side chains); and Class VI: Phe, Trp, Tyr and His (representing aromatic side chains). The classes also include related amino acids such as 3Hyp and 4Hyp in Class I; homocysteine in Class II; 2-aminoacidipic acid, 2-aminopimelic acid, γ-carboxyglutamic acid, β-carboxyaspartic acid, and the corresponding amino acid amides in Class III; ornithine, homoarginine, N-methyl lysine, dimethyl lysine, trimethyl lysine, 2,3-diaminopropionic acid, 2,4-diaminobutyric acid, homoarginine, sarcosine and hydroxylysine in Class IV; substituted phenylalanines, norleucine, norvaline, 2-aminoocetanoic acid, 2-aminoheptanoic acid, statine and β-valine in Class V; and naphthylalanines, substituted phenylalanines, tetrahydroisoquinoline-3-carboxylic acid, and halogenated tyrosines in Class VI.

The peptides of the invention may be synthesized by the solid phase method using standard methods based on either t-butyloxy carbonyl (BOC) or 9-
fluorenylmethoxy-carbonyl (FMOC) protecting groups. This methodology is described by G.B. Fields et al. in *Synthetic Peptides: A User's Guide*, W.M. Freeman & Company, New York, NY, pp. 77-183 (1992). The present peptides may also be synthesized via recombinant techniques well known to those skilled in the art. For example, U.S. Patent No. 5,595,887 describes methods of forming a variety of relatively small peptides through expression of a recombinant gene construct coding for a fusion protein which includes a binding protein and one or more copies of the desired target peptide. After expression, the fusion protein is isolated and cleaved using chemical and/or enzymatic methods to produce the desired target peptide.

The peptides used in the methods of the present invention may be employed in a monovalent state (i.e., free peptide or a single peptide fragment coupled to a carrier molecule). The peptides may also be employed as conjugates having more than one (same or different) peptide fragment bound to a single carrier molecule. The carrier may be a biological carrier molecule (e.g., a glycosaminoglycan, a proteoglycan, albumin or the like) or a synthetic polymer (e.g., a polyalkyleneglycol or a synthetic chromatography support). Typically, ovalbumin, human serum albumin, other proteins, polyethylene glycol, or the like are employed as the carrier. Such modifications may increase the apparent affinity and/or change the stability of a peptide. The number of peptide fragments associated with or bound to each carrier can vary, but from about 4 to 8 peptide fragments per carrier molecule are typically obtained under standard coupling conditions.

For instance, peptide/carrier molecule conjugates may be prepared by treating a mixture of peptides and carrier molecules with a coupling agent, such as a carbodiimide. The coupling agent may activate a carboxyl group on either the peptide or the carrier molecule so that the carboxyl group can react with a nucleophile (e.g., an amino or hydroxyl group) on the other member of the peptide/carrier molecule, resulting in the covalent linkage of the peptide and the carrier molecule. Preferably, the conjugate includes at least one peptide fragment which is not linked to the carrier molecule through an amide bond with the α-
carboxyl group of the C-terminal aromatic amino acid residue of the LipAr-terminated fragment.

For example, conjugates of a peptide coupled to ovalbumin may be prepared by dissolving equal amounts of lyophilized peptide and ovalbumin in a small volume of water. In a second tube, 1-ethyl-3-(3-dimethylamino-propyl)-carboiimide hydrochloride (EDC; ten times the amount of peptide) is dissolved in a small amount of water. The EDC solution was added to the peptide/ovalbumin mixture and allowed to react for a number of hours. The mixture may then dialyzed (e.g., into phosphate buffered saline) to obtain a purified solution of peptide/ovalbumin conjugate. Peptide/carrier molecule conjugates prepared by this method typically contain about 4 to 5 peptide fragments per ovalbumin molecule.

As shown in Example 5, the peptides described herein are degraded enzymatically. Thus, to prolong activity of the peptides for any of the methods described herein, a compound that inhibits the enzymatic degradation of the β1-integrin inhibitor can be coadministered with the β1-integrin inhibitor.

The present invention also provides a composition that includes one or more active agents (i.e., compound such as a peptide) of the invention and one or more pharmaceutically acceptable carriers. The methods of the invention include administering to a patient, preferably a mammal, and more preferably a human, the composition of the invention in an amount effective to produce the desired effect. The agents of the present invention are formulated in pharmaceutical compositions and then, in accordance with the methods of the invention, administered to a mammal, such as a human patient, in a variety of forms adapted to the chosen route of administration. The formulations include those suitable for oral, rectal, vaginal, topical, nasal, opthalmic, or parental (including subcutaneous, intramuscular, intraperitoneal, intratumoral, and intravenous) administration. They may be used as a perfusate for organ transplantation. Preferably, the route of administration is topical or intravenous, or as a perfusate for the preparation of organs for transplantation.

The formulations may be conveniently presented in unit dosage form and
may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing the active agent into association with a carrier which constitutes one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing the active agent into association with a liquid carrier, a finely divided solid carrier, or both, and then, if necessary, shaping the product into the desired formulations.

Formulations suitable for parenteral administration conveniently include a sterile aqueous preparation of the active agent, or dispersions of sterile powders of the active agent, which are preferably isotonic with the blood of the recipient. Isotonic agents that can be included in the liquid preparation include sugars, buffers, and sodium chloride. Solutions of the active agent can be prepared in water, optionally mixed with a nontoxic surfactant. Dispersions of the active agent can be prepared in water, ethanol, a polyol (such as glycerol, propylene glycol, liquid polyethylene glycols, and the like), vegetable oils, glycerol esters, and mixtures thereof. The ultimate dosage form is sterile, fluid, and stable under the conditions of manufacture and storage. The necessary fluidity can be achieved, for example, by using liposomes, by employing the appropriate particle size in the case of dispersions, or by using surfactants. Sterilization of a liquid preparation can be achieved by any convenient method that preserves the bioactivity of the active agent, preferably by filter sterilization. Preferred methods for preparing powders include vacuum drying and freeze drying of the sterile injectable solutions. Subsequent microbial contamination can be prevented using various antimicrobial agents, for example, antibacterial, antiviral and antifungal agents including parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. Absorption of the active agents over a prolonged period can be achieved by including agents for delaying, for example, aluminum monostearate and gelatin.

Formulations of the present invention suitable for oral administration may be presented as discrete units such as tablets, troches, capsules, lozenges, wafers, or cachets, each containing a predetermined amount of the active agent as a powder or granules, as liposomes containing the chemopreventive agent, or as a solution or
suspension in an aqueous liquor or non-aqueous liquid such as a syrup, an elixir, an emulsion, or a draught. Such compositions and preparations typically contain at least about 0.1 wt-% of the active agent. The amount of active agent is such that the dosage level will be effective to produce the desired result (e.g., suppress the development of cancer or tissue destruction by leukocyte mediated inflammation) in the subject.

Nasal spray formulations include purified aqueous solutions of the active agent with preservative agents and isotonic agents. Such formulations are preferably adjusted to a pH and isotonic state compatible with the nasal mucous membranes. Formulations for rectal or vaginal administration may be presented as a suppository with a suitable carrier such as cocoa butter, or hydrogenated fats or hydrogenated fatty carboxylic acids. Ophthalmic formulations are prepared by a similar method to the nasal spray, except that the pH and isotonic factors are preferably adjusted to match that of the eye. Topical formulations include the active agent dissolved or suspended in one or more media such as mineral oil, petroleum, polyhydroxy alcohols, or other bases used for topical pharmaceutical formulations.

Useful dosages of the active agents can be determined by comparing their in vitro activity and the in vivo activity in animals models. Methods for extrapolation of effective dosages in mice, and other animals, to humans are known in the art; for example, see U.S. Patent No. 4,938,949. Generally the concentration of the active agent in a liquid composition will be at least about 0.1 wt-% (wt-%, weight percent, means grams of compound per 100 mL liquid). For adult humans, single dosages for intravenous or topical administration will generally be about 0.005 mg to about 50 mg, and may be administered, for example, about 3 to about 5 times per day, to yield levels of about 0.02 mg to about 200 mg per kg of body weight per day. Suitable doses to be administered, in general, are those that are sufficient to produce the desired result. This will typically not exceed 100 micromoles per kg of body weight per day, and may be much lower.

The tablets, troches, pills, capsules, and the like may also contain one or more of the following: a binder such as gum tragacanth, acacia, corn starch or
gelatin; an excipient such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; a sweetening agent such as sucrose, fructose, lactose or aspartame; and a natural or artificial flavoring agent. When the unit dosage form is a capsule, it may further contain a liquid carrier, such as a vegetable oil or a polyethylene glycol. Various other materials may be present as coatings or to otherwise modify the physical form of the solid unit dosage form. For instance, tablets, pills, or capsules may be coated with gelatin, wax, shellac, or sugar and the like. A syrup or elixir may contain one or more of a sweetening agent, a preservative such as methyl- or propylparaben, an agent to retard crystallization of the sugar, an agent to increase the solubility of any other ingredient, such as a polyhydric alcohol, for example glycerol or sorbitol, a dye, and flavoring agent. The material used in preparing any unit dosage form is substantially nontoxic in the amounts employed. The active agent may be incorporated into sustained-release preparations and devices.

The invention will be further described by reference to the following detailed examples. The examples are meant to provide illustration and should not be construed as limiting the scope of the present invention.

**Examples**

**EXAMPLE 1**

**METHODS**

New Zealand White rabbits (3-5 kg) (Myrtle's Rabbitry, Thompson Station, IN) were shaved and baseline cutaneous blood flow was measured using a laser Doppler blood flow meter with temperature controlled integrated probe (Perimed PF 4001 Stockholm Sweden) (Johnson, Laser-Doppler blood flowmetry; Sherphard AP, Oberg PÅ, eds.; Kluver Academic Publishers, Norwell, MA; pages 121-139, (1990)). The animals were anesthetized using isoflurane inhalation (Abbott Laboratories, North Chicago, IL). Twenty-four gauge catheters (Becton-Dickinson,
Sandy, UT) were placed in ear veins. Three connected brass templates measuring 3 cm x 1 cm x 1 cm each with intervening 5 mm spaces were heated to 100°C and applied to the animals’ backs for 30 seconds to create full thickness burns with intervening 5 mm zones of stasis (Mileski et al., J. Surg. Res., 52, 334-9 (1992); Nwariaku et al., J. Surg. Res., 63, 355-8 (1996)). Analgesia with buprenorphine was administered 0.05 mg/kg every 12 hours (Reckitt & Colman Products Ltd., Hull, England). Blood flow measurements were obtained at the burn sites, the zones of stasis surrounding the burn site, and in unburned sites at 24, 48, and 72 hours post-burn. The number of zones progressing to necrosis was determined at 72 hours. Animals were killed at 72 hours with intravenous pentobarbital 100 mg/kg (Abbott Laboratories, North Chicago, IL).

Trp-9-Tyr is a nine amino acid synthetic peptide of the fibronectin molecule (Peninsula Laboratories, San Carlos, CA) having the sequence WQPRARILY (SEQ ID NO: ____)_. It is stored in powder form at 4°C. Immediately prior to use the powder is dissolved in normal saline to a 10 mg/ml solution for intravenous injection.

There were three experimental groups. Controls (n=10) were given saline (1.0 ml/kg). The first treatment group (n=10) was given the peptide, Trp-9-Tyr (5 mg/kg) immediately after burn, then 3, 6, 12, and 24 hours post-burn. The second treatment group (n=10) received Trp-9-Tyr immediately after burn, then every six hours for 48 hours post-burn.

All animal experiments were approved by and performed in accordance with policies of the Institutional Animal Care and Use Committee of the University of Texas Medical Branch at Galveston.

RESULTS

There were no significant differences in weight change following burn in the three groups. Gross visual observation and laser doppler blood flow measurement of the burn sites were consistent with full thickness injury. Average baseline laser Doppler blood flows of the three groups ranged from 70.69 to 80.56 perfusion units.
**Bloodflow.** Control animals had diminished blood flow in the zone of stasis at 24 hours post-burn (29.97 ± 2.94). There was some recovery at 48 and 72 hours. However, blood flow remained significantly lower than baseline blood flow.

Animals in the 24-hour treatment group had significantly higher blood flow in the zone of stasis than controls at 24 hours post-burn (42.25 ± 3.40). However, there was no difference in perfusion at 48 and 72 hours.

Skin perfusion of the 48-hour treated animals was significantly higher than controls and animals treated for 24 hours at all time points measured. Although blood flow was significantly lower than baseline at 24 hours post-burn (56.23 ± 2.55), perfusion recovered and was not significantly different from baseline at either 48 or 72 hours (Figure 1).

**Tissue Necrosis.** Control animals had 16 of 40 (40%) of zones of stasis progressing to necrosis at 72 hours. Animals receiving Trp-9-Tyr for 24 hours had 7 of 40 (17%) of zones progressing to necrosis, significantly less tissue destruction than controls (p < 0.05 chi-square). Animals treated for 48 hours had 4 of 40 (10%) of zones progressing to necrosis, which was significantly less tissue loss than controls (p < 0.05 chi-square, Mann-Whitney) (Table 1 and Figures 8-10).

| Table 1 |
| Tabulation of zones of stasis progressing to necrosis comparing the 24- and 48 hour-treatment groups to controls. |

<table>
<thead>
<tr>
<th>Number of Animals</th>
<th>Total # Zones</th>
<th>Zones with Necrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>40</td>
</tr>
<tr>
<td>24 hour</td>
<td>10</td>
<td>40</td>
</tr>
<tr>
<td>48 hour</td>
<td>10</td>
<td>40</td>
</tr>
</tbody>
</table>

*Animals in the 24-hour treatment group had 7 of 40 zones (17%) progress to necrosis, significantly fewer zones than controls (p < 0.05 chi-square). Forty-eight hour treatment animals had necrosis in only 4 of 40 zones (10%), significantly less tissue destruction than controls (p < 0.05 chi-square and Mann-Whitney).

DISCUSSION
Emphasis on microvascular injury secondary to leukocyte adherence to the endothelium has been the key to investigation of substances which inhibit inflammation. More recently, the extracellular matrix has become a point of interest for investigation of the inflammatory response to injury. The extracellular matrix macromolecule, fibronectin has reemerged as having a potentially significant role in the pathogenesis of the inflammatory processes.

Synthetic analogues of the RGD domain of fibronectin have been used to prevent acute and chronic experimental liver injury in mice. Animals receiving intravenous RGD mimetics had lower serum levels of liver enzymes and less liver damage by histology than untreated mice following induction of hepatitis from intravenous concanavalin A injection (Bruck et al., *Yale J. Biol. Med.*, 70, 391-402 (1997)).

Synthetic fibronectin peptides derived from the 33-kD carboxyl-terminal heparin-binding domain of fibronectin have demonstrated efficacy in blocking neutrophil accumulation in models of acute inflammation. Two of the more potent synthetic peptides identified thus far are WQPPRARIY (SEQ ID NO:____) and CS-1. Using a rodent model of ischemic brain injury, Yanaka, et al. demonstrated that administration of WQPPRARIY (SEQ ID NO:____) and CS-1 decrease leukocyte accumulation, effectively reduced infarct size, and improved neurological assessment (Yanaka et al., *J. Neurosurg.*, 85, 125-30 (1996); and Yanaka et al., *J. Cereb. Blood Flow Metab.*, 16, 1120-5 (1996)). Synthetic fibronectin peptides block development of inflammatory lesions in salivary glands of TGF-β₁ knock-out mice and restore saliva production (McCartney-Francis et al., *J. Immunol.*, 157:1306-12 (1996)). These peptides blocked leukocyte infiltration into heart and lung tissues of TGF-β₁ knock-out mice, as demonstrated by histopathology. These animals had reduced weight loss and extended life span compared to untreated knock-out mice (Hines et al., *Proc. Natl. Acad. Sci., USA*, 91, 5187-91 (1994)).


Migration of neutrophils into sites of inflammation has been reported to be initiated by a factor released by monocytes (Mileski et al., Circ. Shock, 31, 259-67 (1990)). The synthetic fibronectin peptide, Trp-9-Tyr, has also been demonstrated to inhibit monocyte accumulation via the pathways related to CS-1 (Wahl et al., J. Clin. Invest., 94, 655-62 (1994)). CS-1 interacts with α4β1 integrin expressed on monocytes, altering their function. This suggests that the synthetic fibronectin peptides may inhibit late accumulation of neutrophils into inflammatory tissue.

Other mechanisms of action for the peptide WQPPRARIY (SEQ ID NO:____) have been considered. It may block signal transduction pathways and cytokine presentation. In addition, an α4β1 integrin also serves as a receptor for vascular cell adhesion molecule-1 (VCAM-1), which is expressed on endothelial cells (Wahl et al., J. Clin. Invest., 94, 655-62 (1994)). By interacting with α4β1 integrin, the peptide WQPPRARIY (SEQ ID NO:____) may block leukocyte adhesion to the microvascular endothelium.

In this study, the administration of synthetic fibronectin peptide, Trp-9-Tyr (WQPPRARIY (SEQ ID NO:____)), reduced tissue necrosis following burn injury. These results were somewhat evidenced in the 24-hour treatment group. However, the improvements in blood flow and tissue necrosis were significant in the 48-hour treatment group.

We hypothesize that the difference in blood flow seen at 24 hours between the two treatment groups was due to variation in the dosing schedules. Twenty-hour treatment animals received Trp-9-Tyr immediately, then 3, 6, 12, and 24 hours. The 48-hour treatment group received an 18-hour dose rather than a 3-hour dose. It is possible that the constant 6-hour dosing interval is more effective than an initial bolus of Trp-9-Tyr. Furthermore, the inflammatory process produces tissue destruction for a period longer than 24 hours. By extending the treatment beyond
24 hours, we were able to demonstrate improved blood flow and tissue salvage in the 48-hour group compared to 24-hour group.

In summary, intravenous administration of Trp-9-Tyr improved blood flow in the marginal zones of stasis surrounding burn. The use of the synthetic fibronectin peptide was associated with less tissue destruction following thermal injury.

EXAMPLE 2

Side-Effects of Administration of Peptides in the Treatment of Burns

In addition to mediating responses to injury-mediated inflammation, leukocytes also play an important role in responses to pathogenic infections within tissues. As such, inhibitors of leukocyte adhesion can and have been demonstrated previously to inhibit cellular responses to tissue infection -- in particular soft tissue infections elicited in association with, but not limited to, thermal/cold injury, surgical incisions, injury-mediated trauma, and transplant reperfusion.

Hence, while it is increasingly apparent that leukocytes are involved in the pathogenesis of microvascular injury following infection and inflammation, justifiable concern over the merits of inhibiting leukocyte adherence and possibly increasing susceptibility to infection must be addressed. Much of current knowledge of leukocyte adherence stems from work with patients identified as having leukocyte adherence deficiency (LAD). Patients with LAD are plagued with chronic soft tissue infections.

Moreover, patients are often immuno-compromised, due to ongoing pathological conditions and/or disease, chemotherapeutic and/or cytoxic therapies and/or subsequent to clinical treatment therapies and the like. Thus, it is important that these individuals are not exposed to therapies which further compromise their capability to respond effectively pathological infections.
METHODS

New Zealand white rabbits (1.8-2.5 kg each) were the experimental subjects. Animals were prepared by insertion of a 24-gauge catheter in a marginal ear vein, and clipping of the hair on their dorsa.

Baseline weights, temperatures, hematocrits and WBC counts were obtained. Then the animals were given two sets of paired subcutaneous injections with *S. aureus ATCC 25923*. Following these injections animals were given Trp-9-Tyr as described below. In addition animals were given the antibiotic cefazolin (20 mg/kg intravenously every 8 hours for three doses). Weights, temperature, WBC counts and hematocrits were measured daily for 7 days. Buprenorphine (0.05 mg/kg) was administered for analgesia as determined from a quantitative assessment of pain score. The animals were sacrificed with a lethal intravenous injection of pentobarbital (150 mg/kg) on day 7, and the dorsal skin was dissected from the muscular fascia to allow determination of the incidence of subcutaneous abscess formation and measurement of abscess size by planimetry.

Standard clinical laboratory *S. aureus ATCC 25923* was grown on tryptic soy agar blood plates at 37°C, harvested and resuspended in sterile saline at concentrations of $10^8$ and $10^9$ CFU/ml as determined by A540 measurements. The animals were given two subcutaneous injections (1.0 ml each) of the $10^9$ CFU suspension and two subcutaneous injections (1.0 ml each) of the $10^8$ CFU suspension via a 25-gauge needle and 3.0 ml syringe. Quantitative correlations of bacterial inocula were performed on each day of bacterial preparation. Mean CFU of *S. aureus* were 1.02 +/- 0.21 times the expected concentrations determined by optical density. Statistical analysis of the data was performed by analysis of variance for repeated measures, t test, chi-square test and Kaplan-Meier survival analysis. Significance was assigned to P < 0.05.

**Peptide Administration.** Trp-9-Tyr synthetic peptide (stored in powder form at 4°C) was dissolved in normal saline to a 10 mg/ml solution for intravenous injection immediately before use. There were two treatment groups, control and
peptide treatment. Controls were given saline (1.0 ml/kg). Treatment group was
given the peptide, Trp-9-Tyr (5 mg/kg) immediately after burn, then 3, 6, 12, 24 and
48 hours post-burn.

All animal experiments were approved by and performed in accordance with
policies of the Institutional Animal Care and Use Committee of the University of
Texas Medical Branch at Galveston.

RESULTS

The results of these studies are shown in Figures 2-7. The number of
abscesses formed in treated animals was not greater than that of control animals that
did not receive the β1-integrin inhibitory peptide. In addition, the abscess size in
treated animals was not greater than that observed in controls; and if anything the
abscess size in treated animals was reduced from that observed in controls.

Daily measurements of temperature, body temperature, WBC and hematocrit
were not elevated compared to control animals, as would have been expected if
treatment with β1-integrin inhibitory peptide Trp-9-Tyr had blocked the ability to
response to bacterial infection. In contrast, daily temperature measurements for
peptide treated animals were actually less than controls. In addition, treated animals
not only had a similar number and size of abscess formation, but also had
significantly better weight gain profile than controls. In conclusion, this study
found no evidence that (Trp-9-Tyr) possesses immuno-comprising activity.

EXAMPLE 3

Local Infusion of Two Peptides in Stroke Model

MATERIALS AND METHODS

Adult Sprague-Dawley rats, weighing 300-350 grams, were randomly
assigned to three groups: phosphate buffered saline (PBS) (n=6), PRARI (SEQ ID
NO:___) (n=4), and PRARIY (SEQ ID NO:____) (n=5). The rats were subjected to
1 hour MCAO (middle cerebral artery occlusion) with 48 hours reperfusion. The
peptides, PRARI (SEQ ID NO:_____) and PRARIY (SEQ ID NO:_____) (20 mg/kg in PBS), and the same volume of PBS were continuously infused though extra common artery (local infusion) by an osmotic minipump (0.8 µl/hour) at the time of reperfusion. Neurological deficits were tested at 3, 24, and 48 hours after MCAO, and marked with a grading scale of 0-5 (0, no neurological deficits; 5, severe neurological deficits). Forty-eight hours after reperfusion, the rats were sacrificed and the brains were quickly removed and frozen in powdered dry ice. The coronal cryostat sections, 20 µm thickness, 900 µm interval were used for infarction size measurements (data shown in Figure 12); and 10 µm thick sections through infarction area were taken for immunohistochemical staining to detect leukocyte infiltration (by MPO antibody). Neurological analysis is presented in Figure 13.

RESULTS

The peptide PRARIY (SEQ. ID NO:______) treated rats demonstrated a significant functional improvement at 24 hours and 48 hours after MCAO in comparison with PBS-treated rats; no significant difference between PRARI (SEQ ID NO:_____) and PBS, PRARI (SEQ ID NO:_____) and PRARIY (SEQ ID NO:_____) treated groups was observed. The infarction volume was significantly reduced in PRARIY (SEQ ID NO:______) treated rats when compared with PBS group; no significant difference between PRARI (SEQ ID NO:______) versus PBS and PRARI (SEQ ID NO:______) versus PRARIY (SEQ ID NO:______) treated groups was observed. There was no difference in leukocyte recruitment among the three groups.

DISCUSSION

This experiment was conducted to determine the anti-adhesion effect of the peptide PRARIY (SEQ ID NO:_____) administered via local infusion on brain ischemia/reperfusion injury. The results show that the peptide PRARIY (SEQ ID NO:_____) protects neurons against brain ischemia/reperfusion injury as evidenced
by the smaller infarct size and the rapid reversal of transient neurological deficit. With local infusion of the peptide near the site of injury, at least 80% reduction in infarct size and at least 80% reduction in neurological deficit was observed.

**EXAMPLE 4**

**Equivalence of β1-integrin Inhibitor Peptide to Anti-β1-integrin Antibody**

Soluble peptides or specific anti-β1-integrin antibody was mixed with lymphocytes prior to the start of the adhesion assay. Following a brief (15 minute) preincubation, the cells were added to culture wells containing confluent endothelial that had been activated with cytokines. Following a 30 minute incubation, the lymphocyte/endothelial cultures were washed to remove weakly and nonadherent cells. The data represent the percentage of input cells remaining after this washing step. See Figures 14 and 15. The results indicate that either Trp-9-Tyr or a specific inhibitory anti-integrin antibody can achieve the same level of inhibition of adhesion in this assay.

**EXAMPLE 5**

**Stability and Pharmacokinetics of a Bioactive Peptide**

**METHODS**

*Stability Studies In Vitro* Stability studies were performed in various media, including aqueous solution, rat plasma, human plasma, plasma treated with methanol and rat blood. Solutions of WQPRARIY (SEQ ID NO:____) (concentration range, 10-400 mcg/ml) were incubated at 37 °C for periods up to 4 hours. Aliquots of the plasma incubates, taken frequently at early times, were immediately added to methanol to deactivate enzymes, diluted with water and applied to C-2 cartridges for solid-phase extraction of WQPRARIY (SEQ ID NO:____). After clean up and elution, aliquots of the eluant were analyzed by a validated high-performance liquid chromatographic assay with an UV detector at wavelength of 220 nm following reversed-phase separation on a C-18 column (4.6*100 mm). The mobile phase was ammonium acetate (0.05 M, pH 4.53):
methanol (58:42 v/v) and flow rate was 0.5 ml/minute. Plots of peak area (or height) ratio to internal standard (PX7068) vs. WQQPRARIY (SEQ ID NO:___) concentration were linear from 2-400 mcg/ml in human plasma, rat plasma and rat blood. Aqueous solution samples were directly injected onto the column after incubation.

Pharmacokinetic Study. Four rats (225-280 g) were used in the pharmacokinetic study. The animal protocol was reviewed and approved prior to animal experimentation by the University of Minnesota’s committee on animal use and care. The femoral artery and vein were cannulated for drug sampling and administration, respectively. This was a two-phase crossover study. The first phase was a low-dose (5 mg/min-kg) infusion for 15 minutes. Immediately after this, the animals received a high-dose (10 mg/kg/min) for 15 minutes, followed by a washout period. Intravenous formulations of WQQPRARIY (SEQ ID NO:___) were prepared by dissolving an accurately weighed amount of the peptide in physiological saline. Blood samples were collected from femoral artery at 6, 9, 12 and 15 min after dose initiation in both phases. Additionally, from 3 to 6 samples were drawn in the 5 minutes following the termination of the high infusion rate. Blood samples were immediately treated with methanol to inactivate enzymes, and processed using the same procedure described for the plasma study performed in vitro.

The blood samples from the pharmacokinetic study were analyzed by a validated high-performance liquid chromatographic assay with an UV detector at wavelength of 220 nm. Mobile phase was as described above. Within-run and between-run variability of the assay was characterized by CVs of less than 10%. The average analytical recovery was 70 ± 4.2%. Similar results were found in rat plasma and rat blood. The inclusion of quality control samples demonstrated accuracy, precision, and reproducibility of the method.
RESULTS

The peptide WQPRARIY (SEQ ID NO:____) was stable in PBS (0.1 M, pH 7.4) and in water over 4 hours of incubation at 37°C but markedly unstable in rat plasma and human plasma. At initial concentrations of 10 to 40 mcg/ml in human plasma the peptide exhibits an effective half-life of less than 3 minutes; at 100 and 400 mcg/ml it exhibited half-lives of 5 minutes and 15 minutes, respectively (Figure 17).

Although not plotted here, similar degradation rates were seen in rat plasma. Chromatographic peaks of several degradation products were observed in both human and rat plasma and these exhibited longer half lives than WQPRARIY (SEQ ID NO:____). In plasma treated with methanol, the peptide was stable over the 4-hour incubation at 37°C.

The blood concentration-time data obtained in the constant-rate infusion studies were subjected to compartment analysis using SAAM II (Program for Kinetic Analysis, version 1.1.1). It was assumed that WQPRARIY (SEQ ID NO:____) obeyed a one-compartment model with one elimination pathway of the Michaelis-Menten type. Parameters determined in the analysis were V (liter), the maximum velocity of metabolism (V_max), and K_m. The mean and individual pharmacokinetics parameters are provided in Table 2. The mean plasma concentration versus time profiles of WQPRARIY (SEQ ID NO:____) during the two-phase infusion study are depicted in Figure 18.
Table 2. Individual and Mean (SD) Pharmacokinetic Parameters in Rats.

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<tr>
<th>Parameter</th>
<th>Rat</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
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<tr>
<td>V (L/kg)</td>
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<td>0.71</td>
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<tr>
<td>Vmax (mg/min-kg)</td>
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<td>15.5</td>
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<tr>
<td>Km (mg/L)</td>
<td>17.5</td>
<td>17.9</td>
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</table>

Based on preliminary studies not reported here, the half-life of this peptide *in vivo* was estimated to be less than two minutes in concentrations produced by doses of 10 to 20 mg/kg. The infusion study was performed in order to obtain estimates of clearance at steady state, and to characterize saturability of elimination, a crossover study with two infusion rates was employed.

Blood concentrations of WQPPRARIY (SEQ ID NO:____) approached steady state during infusion period, and mean blood concentration (SD) during the low and high dose phases were 11.4 (0.84) and 36.33 (4.0) μg/ml, respectively. This lack of proportionality between steady-state concentrations and infusion rates is a characteristic of Michaelis-Menten elimination kinetics, and is consistent with the results of the *in vitro* studies. Parameters are calculated using SAAM II in three rats. The mean apparent volume of distribution (SD) is 0.77 (0.27) L, which is
similar to the volume of total body water in the rat, suggesting significant
distribution of the peptide into tissues.

It should be noted that if two sets of steady-state blood concentrations at two
infusion rates are available, one can normally estimate the operative Michaelis-
Menten parameters that describe the saturability of metabolism of the infused
therapeutic agent. In order to estimate a volume of distribution, changing blood
levels within the animal need to be determined, a challenge for a compound with a
short effective half-life. Thus, data from one of the four animals, which afforded
only 2 measurable samples in the post-infusion period, did not allow convergence in
SAAM II and are not included in the statistical analysis and parameter summary.

The short half-life of WQPPRARIY (SEQ ID NO:____) in plasma in vitro
demonstrates rapid enzymatic degradation that does not require perfusion of the
peptide through organ systems in order for metabolism to occur. It is of interest that
when the half-life of the peptide is calculated from means of the estimates of the
Michaelis-Menten parameters and the volume of distribution, these are found to
range from 1.1 to 15.5 min for concentrations ranging from 10 to 400 mg/Liter.
These values are consistent with those found in the studies in vitro, and seem to
suggest that circulating enzymes are primarily responsible for the disposition of FN-
C/H-V.

Finally, a pharmacokinetic study of the rapidly metabolized peptide
WQPPRARIY (SEQ ID NO:____) was performed in vivo using high dose-short
infusion periods at two rates. Michaelis-Menten parameters, suggesting saturation
of blood-borne peptidases were estimated. The results are consistent with the
degradation of the peptide incubated in rat and human plasma, and suggest efficient
processing by peptidases. Although not identified, intermediates that resulted from
the degradation of WQPPRARIY (SEQ ID NO:____) were observed in the studies
in vitro. However, their role in inhibiting the cell adhesion ascribed to the parent
peptide is unknown.
DISCUSSION

Transient cerebral ischemia and associated brain injury may be mediated by several factors, including inflammatory processes (Hallenbeck et al., Stroke, 17, 246-253 (1986)). Leukocyte infiltration into ischemic tissue is a pathophysiological response, which often further aggravates ischemic injury by attenuating microvascular blood flow, and releasing chemical mediators such as free oxygen radicals (Kochanek et al., Stroke, 23, 1367-1379 (1992); and Matsuo et al., J. Cereb. Blood Flow Met., 15, 941-947 (1995)). Cell adhesion molecules play important roles in leukocyte-endothelial interactions: the selectins (Lasky, Science, 258, 964-969 (1992)), the integrins, and the immunoglobulin superfamilies (Springer, Nature, 346, 425-434 (1990)). Integrins which contain β1 subunits usually are associated with mediating adhesion to extracellular matrix constituents (Springer, Nature, 346, 425-434 (1990)) whereas β2 integrins are largely involved in cell-cell interactions. One of these extracellular matrix macromolecules is fibronectin, which is found in plasma, cell matrix, and on the cell surface. These molecules can support leukocyte adhesion to endothelial cells (Akiyama et al., Adv. Enzymol., 57, 1-57 (1987)).

Fibronectin possesses multiple domains recognized by integrins, including arginyl-glycyl-aspartic acid (RGD). The latter interacts selectively with α5β1 integrin, and the alternately spliced connecting segment domain (CS-1) which is recognized selectively by α4β1 integrin (Akiyama et al., Adv. Enzymol., 57, 1-57 (1987); and Guan et al., Cell, 60, 53-61 (1990)). Over the last few years several novel (nonRGD/nonCS-1) bioactive peptides from fibronectin that: a) antagonize leukocyte adhesion of activated lymphocytes and monocytes in vitro when used as soluble antagonists and b) show efficacy for improved outcomes in several in vivo animal models of chronic and acute inflammation when administered intravenously. These models include bacterial cell wall-induced arthritis in rats, models of autoimmune disease such as TGF-β -/- mice, and reperfusion injury in rat transient cerebral ischemia and in rabbit burn models (Hines et al., Proc. Natl. Acad. Sci., USA, 91, 5187-5191 (1994); Wahl et al., J. Clin. Invest., 94, 655-662
(1994); and unpublished data).

Peptide WQPRARIY (SEQ ID NO:___) has been characterized over the last several years with regard to structure/activity relationships and its molecular target of this peptide. The studies have shown that this peptide inhibits adhesion by a unique molecular mechanism. The minimum structure of the peptide (WQPRARIY (SEQ ID NO:___)) needed to inhibit adhesion has been identified as the last two residues of this peptide (IY) (International Publication No. WO 99/37669). Secondly, this dipeptide has been shown to represent a more generalized structural motif that can effectively inhibit adhesion, where the amino acids consist of a branched hydrophobic residue plus an aromatic residue). Finally, we have demonstrated by several approaches that this structural motif represents a novel peptide based generalized inhibitor of β₁ integrin mediated cell adhesion.

Sequence Free Text

The following are all peptides:

WQPRARIY (SEQ ID NO:___)
QPPRAAIY (SEQ ID NO:___)
WQPPAARIY (SEQ ID NO:___)
AQPRARIY (SEQ ID NO:___)

WAPPRAIY (SEQ ID NO:___)
WQPPDADIY (SEQ ID NO:___)
ARITGYIIY (SEQ ID NO:___)
RARITGYIIY (SEQ ID NO:___)
PRQAWRPIY (SEQ ID NO:___)

RPAPQRWIIY (SEQ ID NO:___)
ARITGYIIY (SEQ ID NO:___)
PRARIY (SEQ ID NO:___)
PRARI (SEQ ID NO:___)
RARIIY (SEQ ID NO:___)

ARIY (SEQ ID NO:___).
Although the invention has been disclosed with reference to its preferred embodiments, from reading this description those of skill in the art may appreciate changes and modification that may be made which do not depart from the scope and spirit of the invention as described above and claimed hereafter. All references, patents, and patent applications cited herein are incorporated herein by reference in their entirety as if individually incorporated.
WHAT IS CLAIMED IS:

1. A method of inhibiting inflammatory leukocyte mediated destruction of tissue in a patient, the method comprising administering to the patient a composition comprising a β1-integrin inhibitor.

2. The method of claim 1 wherein the β1-integrin inhibitor is a peptide comprising a C-terminal LipAr motif.

3. The method of claim 2 wherein the β1-integrin inhibitor is a peptide comprising an amino acid sequence selected from the group consisting of WQPPARIIY (SEQ ID NO:___), QPPRAAIY (SEQ ID NO:___), WQPPAAIY (SEQ ID NO:___), AQPPRAIY (SEQ ID NO:___), WAPPRARIIY (SEQ ID NO:___), WQPPDADIY (SEQ ID NO:___), ARITGYIIY (SEQ ID NO:___), RARITGYIY (SEQ ID NO:___), PRQAWRPIY (SEQ ID NO:___), RPAPQRWIIY (SEQ ID NO:___), ARITGYIIY (SEQ ID NO:___), PRARIY (SEQ ID NO:___), RARIY (SEQ ID NO:___), ARIY (SEQ ID NO:___), and RIY.

4. The method of claim 1 wherein the composition further includes a pharmaceutically acceptable carrier.

5. The method of claim 1 wherein the inflammatory leukocyte mediated destruction of tissue occurs as a result of CNS ischemic injury, myocardial infarction, angioplasty, surgical incisions, injury-related trauma, transplant reperfusion, or a combination thereof.

6. The method of claim 1 wherein the inflammatory leukocyte mediated destruction of tissue occurs as a result of exposure to heat, cold, light, electricity, chemicals, or a combination thereof.
7. A method of treating a stroke patient, the method comprising administering to the patient a composition comprising a β1-integrin inhibitor in an amount effective to reduce infarct size, reduce neurological deficit, or both.

8. The method of claim 7 wherein the composition is administered locally.

9. The method of claim 8 wherein the β1-integrin inhibitor is administered in an amount effective to reduce the infarct size by at least about 80%.

10. The method of claim 8 wherein the β1-integrin inhibitor is administered in an amount effective to reduce the neurological deficits by at least about 80%.

11. The method of claim 7 wherein the β1-integrin inhibitor is a peptide comprising a C-terminal LipAr motif.

12. The method of claim 11 wherein the β1-integrin inhibitor is a peptide comprising an amino acid sequence selected from the group consisting of WQPPARAIY (SEQ ID NO:___), QPPRAAIY (SEQ ID NO:___), WQPPAARIIY (SEQ ID NO:___), AQPPRARIY (SEQ ID NO:___), WAPPRARIY (SEQ ID NO:___), WQPPDADIY (SEQ ID NO:___), ARITGYIY (SEQ ID NO:___), RARITGYIY (SEQ ID NO:___), PRQAWRPRIY (SEQ ID NO:___), RPAPQRWYIY (SEQ ID NO:___), ARITGYIY (SEQ ID NO:___), PRARIY (SEQ ID NO:___), RARIY (SEQ ID NO:___), ARIY (SEQ ID NO:___), and RIY.

13. A method of treating a patient having a burn-type injury, the method comprising administering a composition comprising a β1-integrin inhibitor
in an amount effective and over a period of time effective to reduce
leukocyte-mediated tissue destruction.

14. The method of claim 13 wherein the β1-integrin inhibitor is a peptide
comprising a C-terminal LipAr motif.

15. The method of claim 14 wherein the β1-integrin inhibitor is a peptide
comprising an amino acid sequence selected from the group consisting of
WQPPRARIIY (SEQ ID NO:___), QPPRAAIY (SEQ ID NO:___),
WQPPAARIY (SEQ ID NO:___), AQPPRARIY (SEQ ID NO:___),
WAPPRARIY (SEQ ID NO:___), WQPPDADIY (SEQ ID NO:___),
ARITGYIY (SEQ ID NO:___), RARITGYIY (SEQ ID NO:___),
PRQAWRPIY (SEQ ID NO:___), RPAPQRWYIY (SEQ ID NO:___),
ARITGYII (SEQ ID NO:___), PRARIY (SEQ ID NO:___), RARIY (SEQ
ID NO:___), ARIY (SEQ ID NO:___), and RIY.

16. The method of claim 15 wherein the β1-integrin inhibitor is a peptide
comprising the amino acid sequence WQPPRARIIY (SEQ ID NO:___).

17. The method of claim 13 wherein the period of time is at least 1 hour.

18. The method of claim 17 wherein the period of time is at least 24 hours.

19. The method of claim 18 wherein the period of time is at least 48 hours.

20. The method of claim 13 wherein the composition is administered
periodically over a predetermined period of time.

21. A method of treating a burn patient, the method comprising maintaining a
composition comprising an effective amount of a β1-integrin inhibitor on a
burn-type injury for a period of time effective to reduce leukocyte-mediated tissue destruction and achieve a desired degree of healing.

22. The method of claim 21 wherein the β1-integrin inhibitor is a peptide comprising a C-terminal LipAr motif.

23. The method of claim 22 wherein the β1-integrin inhibitor is a peptide comprising an amino acid sequence selected from the group consisting of WQPPRARIIY (SEQ ID NO: ___), QPPRAAIY (SEQ ID NO: ___), WQPPAARIY (SEQ ID NO: ___), AQPPRARIIY (SEQ ID NO: ___), WAPPRARIIY (SEQ ID NO: ___), WQPPDAIIY (SEQ ID NO: ___), ARITGYIIY (SEQ ID NO: ___), RARITGYIIY (SEQ ID NO: ___), PRQAWRIPIY (SEQ ID NO: ___), RPAPQRWIIY (SEQ ID NO: ___), ARITGYIIY (SEQ ID NO: ___), PRARIY (SEQ ID NO: ___), RARIY (SEQ ID NO: ___), ARIY (SEQ ID NO: ___), and RIY.

24. The method of claim 23 wherein the β1-integrin inhibitor is a peptide comprising the amino acid sequence WQPPRARIIY (SEQ ID NO: ___).

25. A method of treating a cancer patient, the method comprising administering to the patient a composition comprising a β1-integrin inhibitor in an amount effective to inhibit one or more of angiogenesis, cancer cell metastasis, cancer cell motility, or cancer cell migration.

26. The method of claim 25 wherein the β1-integrin inhibitor is a peptide comprising a C-terminal LipAr motif.

27. The method of claim 26 wherein the β1-integrin inhibitor is a peptide comprising an amino acid sequence selected from the group consisting of WQPPRARIIY (SEQ ID NO: ___), QPPRAAIY (SEQ ID NO: ___),
28. A method of treating a cancer patient, the method comprising administering to the patient a composition comprising a $\beta_1$- integrin inhibitor in an amount effective to induce programmed cell death in cancerous tissue or restore normal cellular phenotype to cancerous tissue.

29. The method of claim 28 wherein the $\beta_1$- integrin inhibitor is a peptide comprising a C-terminal LipAr motif.

30. The method of claim 29 wherein the $\beta_1$- integrin inhibitor is a peptide comprising an amino acid sequence selected from the group consisting of WQPPRARIY (SEQ ID NO:___), AQPRAIY (SEQ ID NO:___), WQPPRARIY (SEQ ID NO:___), AQPRAIY (SEQ ID NO:___), WQPPRARIY (SEQ ID NO:___), WQPPRARIY (SEQ ID NO:___), AQPRAWPY (SEQ ID NO:___), RPAPQRWY (SEQ ID NO:___), ARITGYIY (SEQ ID NO:___), RARITGYIY (SEQ ID NO:___), RARIY (SEQ ID NO:___), ARIY (SEQ ID NO:___), and RIY.

31. The method of claim 28 further comprising administering a compound that inhibits the enzymatic degradation of the $\beta_1$- integrin inhibitor.
A method of treating a patient for osteoporosis, the method comprising administering to the patient a composition comprising a β1-integrin inhibitor in an amount effective to inhibit osteoclast adhesion and bone resorption.

The method of claim 32 wherein the β1-integrin inhibitor is a peptide comprising a C-terminal LipAr motif.

The method of claim 33 wherein the β1-integrin inhibitor is a peptide comprising an amino acid sequence selected from the group consisting of WQPPRARIY (SEQ ID NO:___), QPPRAAIY (SEQ ID NO:___), WQPAAARIY (SEQ ID NO:___), AQPPRARIIY (SEQ ID NO:___), WAPPRARIIY (SEQ ID NO:___), WQPPDADIY (SEQ ID NO:___), ARITGYIY (SEQ ID NO:___), RARITGYIY (SEQ ID NO:___), PRQAWRPIY (SEQ ID NO:___), RPAPQRWRIY (SEQ ID NO:___), ARITGYIY (SEQ ID NO:___), PRARIY (SEQ ID NO:___), RARIY (SEQ ID NO:___), ARIY (SEQ ID NO:___), and RIY.

A method of peripheralizing stem cells, the method comprising administering to a patient a composition comprising a β1-integrin inhibitor.

The method of claim 35 wherein the β1-integrin inhibitor is a peptide comprising a C-terminal LipAr motif.

The method of claim 36 wherein the β1-integrin inhibitor is a peptide comprising an amino acid sequence selected from the group consisting of WQPPRARIY (SEQ ID NO:___), QPPRAAIY (SEQ ID NO:___), WQPAAARIY (SEQ ID NO:___), AQPPRARIIY (SEQ ID NO:___), WAPPRARIIY (SEQ ID NO:___), WQPPDADIY (SEQ ID NO:___), ARITGYIY (SEQ ID NO:___), RARITGYIY (SEQ ID NO:___), PRQAWRPIY (SEQ ID NO:___), RPAPQRWRIY (SEQ ID NO:___),
ARITGYIIY (SEQ ID NO:____), PRARIY (SEQ ID NO:____), RARIY (SEQ ID NO:____), ARIY (SEQ ID NO:____), and RIIY.

38. A composition comprising β1-integrin inhibitor and a pharmaceutically acceptable carrier.

39. The composition of claim 38 wherein the β1-integrin inhibitor is a peptide comprising a C-terminal LipAr motif.

40. The composition of claim 39 wherein the β1-integrin inhibitor is a peptide comprising an amino acid sequence selected from the group consisting of WQPPRARIIY (SEQ ID NO:____), QPPRAAIY (SEQ ID NO:____), WQPPAARIY (SEQ ID NO:____), AQPPRARIIY (SEQ ID NO:____), WAPPRARIIY (SEQ ID NO:____), WQPPDAIIY (SEQ ID NO:____), ARITGYIIY (SEQ ID NO:____), RARITGYIIY (SEQ ID NO:____), PRQAWRPRIY (SEQ ID NO:____), RPAPQRWIIY (SEQ ID NO:____), ARITGYIIY (SEQ ID NO:____), PRARIY (SEQ ID NO:____), RARIY (SEQ ID NO:____), ARIY (SEQ ID NO:____), and RIIY.
Marginal Zone Doppler Blood Flow

* indicates p<0.05 (compared to Baseline)
+ indicates p<0.05 (compared to Control)

Baseline  24 hours  48 hours  72 hours

FIG. 1
Doppler Blood Flow in Marginal Zones

* indicates p<0.05 (compared to control)

- ■ Control  (n=10)
- □ 24 Hr Tx  (n=10)
- ○ 48 Hr Tx  (n=10)

Percent Change from Baseline

Baseline  24 Hours  48 Hours  72 Hours

FIG. 2
Rabbit Weight
(* p<0.05 t-test)

Percent Change from Baseline

Days

FIG. 3
Body Temperature (°C, p<0.05 t-test)

Percent Change from Baseline

Days

FIG. 5
### Abscess Formation at Day 7

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<td>2</td>
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<tr>
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**FIG. 11**
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<th>Abscess Size at Day 7 (Area=square cm)</th>
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<tr>
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**FIG. 12**
Infarction size

I.A. (local) infusion

% of contralateral hemisphere

Group value

FIG. 13

PBS N=6

PRAR I N=4

NY N=5

(27±3±3.03)

(9.5±1.67)

(3.4±3.6)

FIG. 13