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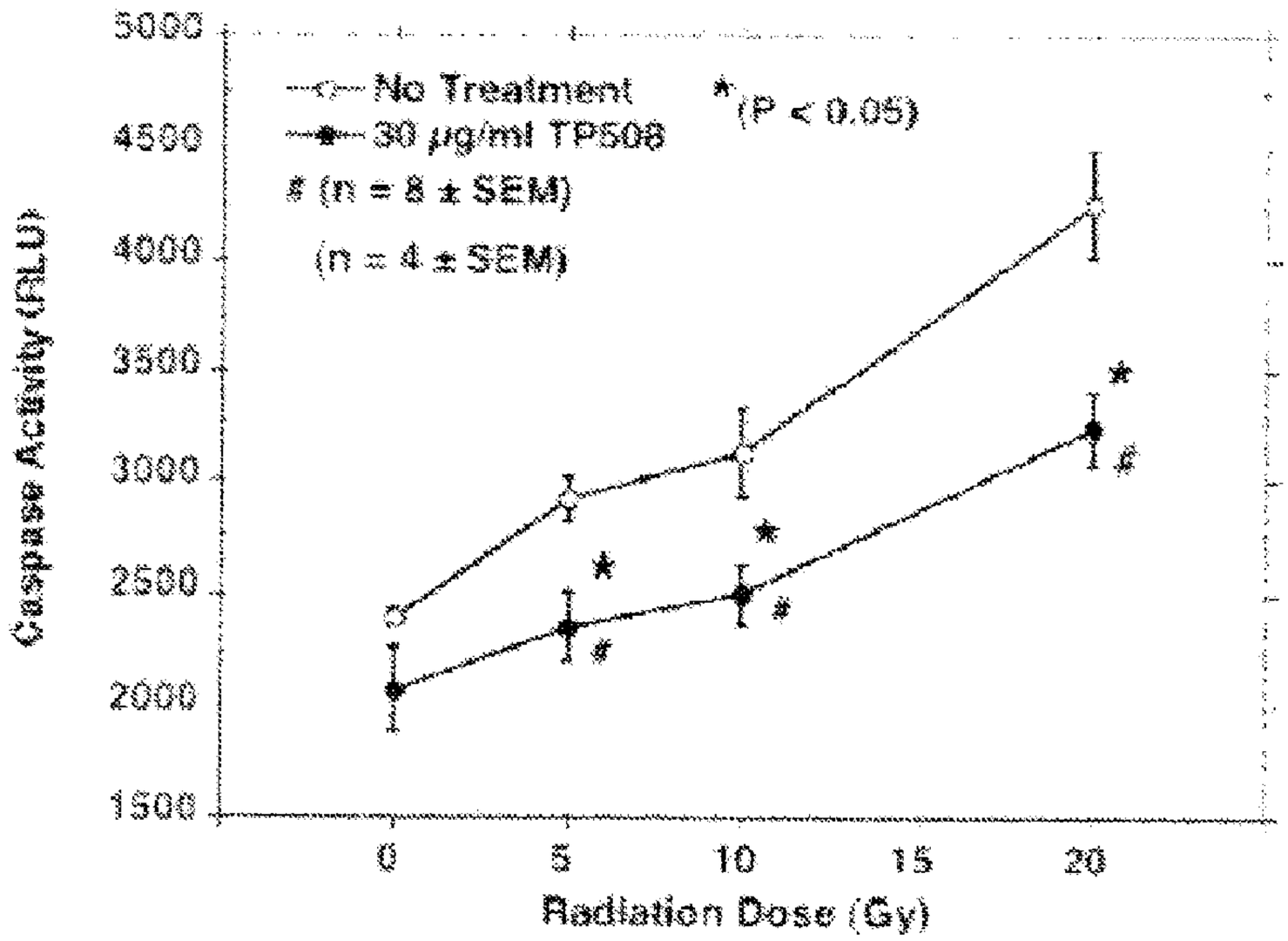

Figure 1.

(57) Abrégé/Abstract:
The present invention is directed to methods of reducing mortality in a subject exposed to a lethal dose of radiation comprising administering to the subject an effective amount of a thrombin peptide derivative described herein. Also included are methods of reducing the risk of developing bacterial, fungal or viral systemic infection a subject who is exposed or not exposed to radiation, methods of treating a subject with traumatic injury, dermal injury and/or bum injury who is also exposed to radiation, methods of reducing radiation related injury in a subject undergoing radiation therapy, methods of reducing the risk of developing a radiation induced illness, and methods of promoting the healing of a wound that is caused by radiation exposure and/or has been exposed to radiation. These methods comprising administering to the subject an effective amount of a thrombin peptide derivative described herein.

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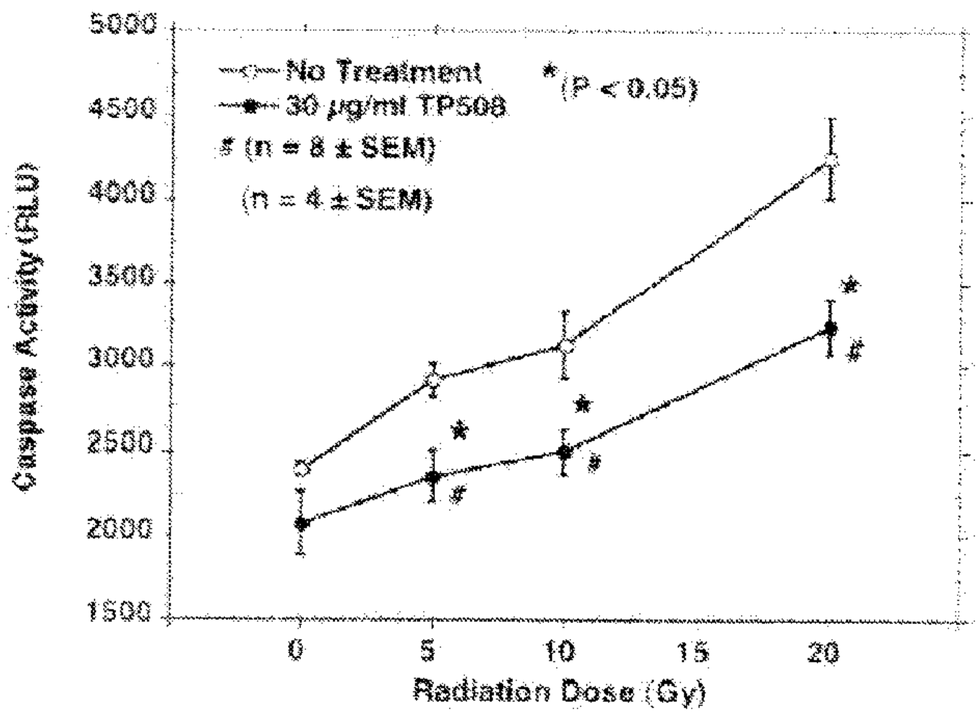

Figure 1.

(57) Abstract: The present invention is directed to methods of reducing mortality in a subject exposed to a lethal dose of radiation comprising administering to the subject an effective amount of a thrombin peptide derivative described herein. Also included are methods of reducing the risk of developing bacterial, fungal or viral systemic infection a subject who is exposed or not exposed to radiation, methods of treating a subject with traumatic injury, dermal injury and/or bum injury who is also exposed to radiation, methods of reducing radiation related injury in a subject undergoing radiation therapy, methods of reducing the risk of developing a radiation induced illness, and methods of promoting the healing of a wound that is caused by radiation exposure and/or has been exposed to radiation. These methods comprising administering to the subject an effective amount of a thrombin peptide derivative described herein.

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METHODS OF MITIGATING EFFECTS OF RADIATION AND REDUCING THE RISK OF SYSTEMIC INFECTION

RELATED APPLICATIONS

This application claims priority to US Provisional Patent Application No. 61/354067, filed June 11, 2010, the contents of which are incorporated herein in their entirety by reference.

BACKGROUND OF THE INVENTION

With increasing threat of a nuclear detonation, it is essential to develop new countermeasures that can be delivered post-exposure to protect civilians and immediate care providers. Further urgency is mandated by the realization that a combination of radiation with traumatic injury, dermal injury or burns can be up to ten times more lethal than radiation alone. A detonation will injure thousands of people, who, without an effective countermeasure for combined radiation injury, will likely die from what should have been a sub-lethal dose of radiation.

In addition, more than 50% of all cancer patients undergo some degree of radiation therapy. It is well known that radiation therapy affects adjacent normal tissue, often preventing closure of surgical wounds and leading to later breakdown of skin or formation of chronic ulcers that fail to heal.

Currently, there are no products that have been approved for mitigating effects of radiation on individuals after radiation exposure. A number of potential products that are being evaluated only target a particular aspect of radiation combined injury, and therefore, have limited efficacies. For radiotherapy related injuries, most treatments are largely based on good wound

care with the use of standard antibiotics and wound dressings with surgical repair of larger ulcerated or non-healing areas. The only FDA approved radiotherapy protective agent, Amifostin, however, is required to be injected into adjacent tissues prior to fractionated radiotherapy. Therefore, a need exists for new methods for preventing and treating radiation induced injuries resulted from accidental radiation exposure or radiotherapy.

SUMMARY OF THE INVENTION

Applicants have discovered that post-exposure injection of the thrombin peptide derivative TP508 can increase survival time and delay onset of septic bacterial growth in mice that were exposed to a lethal dose of gamma irradiation (Examples 3 and 4). In addition, either topical treatment or systemic injection of the thrombin peptide derivative TP508 can promote healing of an open dermal wound in mice that were exposed to radiation (Example 5).

The present invention is directed to a method of reducing the risk of mortality or extending the life expectancy (by, e.g., at least 5%, 10%, 20%, 25%, 50%, 75% or 100%) in a subject exposed to a lethal dose of radiation, or to a dose of radiation that when combined with injury would be lethal, comprising administering to the subject an effective amount of a thrombin peptide derivative comprising Asp-Ala-R, wherein R is a serine esterase conserved sequence.

In another embodiment, the present invention is directed to a method of reducing the risk of developing systemic bacterial, fungal or viral infection in a subject exposed to radiation. The method comprises administering to the subject an effective amount of a thrombin peptide

derivative comprising Asp-Ala-R, wherein R is a serine esterase conserved sequence.

In another embodiment, the present invention is directed to a method of treating a subject with traumatic injury, dermal injury and/or burn injury who is also exposed to radiation, comprising administering to the subject an effective amount of a thrombin peptide derivative, wherein the thrombin peptide derivative comprises Asp-Ala-R, wherein R is a serine esterase conserved sequence.

The present invention is also directed to a method of reducing radiation related injury in a subject undergoing radiation therapy, comprising administering to the subject an effective amount of a thrombin peptide derivative, wherein the thrombin peptide derivative comprises Asp-Ala-R, wherein R is a serine esterase conserved sequence.

In another embodiment, the present invention is directed to a method of reducing the risk of developing a radiation induced illness in a subject undergoing radiation therapy, comprising administering to the subject an effective amount of a thrombin peptide derivative, wherein the thrombin peptide derivative comprises Asp-Ala-R, wherein R is a serine esterase conserved sequence.

In another embodiment, the present invention is directed to a method of promoting healing of a wound on a subject that was caused by radiation exposure and or has been exposed to radiation, comprising administering to the wound an effective amount of a thrombin peptide derivative, or comprising administering an effective amount of a thrombin peptide derivative systemically post radiation exposure, wherein the thrombin peptide derivative comprises Asp-Ala-R, wherein R is a serine esterase conserved sequence.

In yet another embodiment, the present invention is directed to a method of reducing the risk of developing bacterial, fungal or viral infection in the blood of a subject that has not been exposed to radiation and that may be at risk of developing bacterial, fungal or viral infection in the blood. The method comprises administering to the subject an effective amount of a thrombin peptide derivative comprising Asp-Ala-R, wherein R is a serine esterase conserved sequence.

The present invention is also directed to the use of a thrombin peptide derivative for reducing the risk of mortality in a subject exposed to a lethal dose of radiation, reducing the risk of developing bacterial, fungal or viral infection in a subject exposed to radiation, treating a subject with traumatic injury, dermal injury and/or burn injury who is also exposed to radiation, reducing radiation related injury in a subject undergoing radiation therapy, reducing the risk of developing a radiation induced illness in a subject undergoing radiation therapy; promoting healing of a wound on a subject that was caused by radiation exposure and/or has been exposed to radiation, wherein the thrombin peptide derivative comprises Asp-Ala-R, wherein R is a serine esterase conserved sequence. For the use for promoting healing of a wound on a subject that was caused by radiation exposure and/or has been exposed to radiation.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graph that shows caspase activity measured in human microvascular endothelial cells (HMVEC) exposed to 0, 5, 10 or 20 Gy of radiation and treated with saline or 30 ug/ml TP508.

Figure 2 is a graph that shows percent of mice surviving on different days after sustaining 8 Gy radiation exposure and a dermal excision, who have also received treatment with saline placebo or TP508 delivered topically or intravenously.

Figure 3 is a graph that shows percent of mice surviving at different days after receiving 12 Gy radiation exposure and a single post-exposure bolus dose of either saline or TP508.

Figure 4 is a bar graph that shows the number of live bacteria (CFU) in the blood of mice 6 and 7 days after exposure to 0 or 12 Gy radiation and either a saline placebo or TP508 injection.

Figure 5 is a bar graph that shows the rate of linear wound healing (measured as mm/day) in mice at days 0-5 and 5-16 post-exposure to 0 or 8 Gy irradiation and either saline placebo or TP508 applied topically or by intravenous injection.

Figure 6 is a panel with two bar graphs. The bar graph in panel A shows serum IL-6 levels (ng/ml) measured 11 days post-irradiation in mice irradiated at 0 and 8 Gy and treated with either saline placebo (P) or TP508 administered either topically (TPt) or intravenously (TPiv). The bar graph in panel B shows serum IL-6 levels (ng/ml) measured 7 days post-irradiation in mice irradiated at 0 and 12 Gy and treated with either saline placebo (P) or TP508 administered either topically (TPt) or intravenously (TPiv).

Figure 7 is a bar graph showing fold increase in the sprouting area after 5 days of aortic explant culture. The aortas were isolated from mice 24 hours after exposure to 0, 3, 8 or 10 Gy radiation and treatment with either saline placebo or TP508 administered intravenously.

DETAILED DESCRIPTION OF THE EMBODIMENTS

The present invention is directed to methods of reducing the adverse effects of radiation exposure in a subject comprising administering to the subject an effective amount of a thrombin peptide derivative described therein. Radiation exposure can, for example, result from nuclear

detonation, nuclear weaponry, accidental radiation exposure (such as due to accidents at nuclear reactors or inadequate protection from radiation source) or radiation therapy. Low-dose radiation exposure can be an occupational hazard affecting airline workers and astronauts; workers at nuclear power and nuclear fuel processing plants; research laboratory workers; and uranium miners. In addition, medical diagnostic tests, such as X-rays, can be a source of low-level radiation exposure for the general public.

The present invention is also directed to methods of reducing the risk of developing bacterial, fungal or viral infection in the blood in the subjects who have not been exposed to radiation, comprising administering to the subject an effective amount of a thrombin peptide derivative described herein. The infection can enter the bloodstream as a complication of diseases, such as pneumonia or meningitis, during surgery (especially when it involves mucous membranes such as the gastrointestinal tract), or due to catheters and other foreign bodies entering the arteries or veins (including intravenous drug abuse). In addition, individuals suffering from pulmonary conditions, inflammatory bowel disease and systemic inflammatory response syndrome (SIRS), or individuals who are immunocompromised are also at the risk of blood infection. In the hospital, indwelling catheters are a frequent cause of blood infections because they provide a means by which bacteria normally found on the skin can enter the bloodstream. Other causes of blood infections include dental procedures (occasionally including simple tooth brushing), herpes (including herpetic whitlow), urinary tract infections, peritonitis, *Clostridium difficile* colitis, intravenous drug use, and colorectal cancer. Blood infections may also be a consequence of oropharyngeal, gastrointestinal or genitourinary surgery or exploration. An immune response to blood infection can lead to sepsis and septic shock, which have a relatively high mortality rate. In the methods described herein, the peptide of the present

invention can be administered in combination with an antibiotic.

In one aspect, the present invention is directed to a method of reducing the risk of mortality in a subject exposed to a lethal dose of radiation, comprising administering to the subject an effective amount of a thrombin peptide derivative described herein.

A lethal dose of radiation is a dose that would cause death in half of the tested subjects in 10 days, *i.e.* LD_{50} in 10 days. The lethal dose depends on the identity of the subject. For example, a lethal dose for a human is about 3.5 Gy or greater and a lethal dose for a mouse is about 12 Gy or greater.

The subject who is exposed to a lethal dose of radiation may also have additionally sustained traumatic injury, dermal injury and/or burn injury.

A "burn injury" is a type of skin injury caused by heat, electricity, chemicals, light, radiation, friction or heat. Burn injury caused by radiation exposure, such as in the event of nuclear detonation, includes, for example, thermal burns from infrared heat radiation, beta burns from shallow ionizing beta radiation, and gamma burns from highly penetrating gamma radiation. The burn injury can be first-degree, second-degree or third-degree burn. Various percentage of total body surface area (TBSA) may be affected by the burn injury. For example, less than 1%, greater than 1%, greater than 5%, greater than 10%, greater than 15%, greater than 20%, greater than 30%, greater than 40%, greater than 50%, or 1-10%, 10-20%, 20-30%, 30-40%, 40-50% or 50-70% of TBSA was affected by the burn injury.

A "traumatic injury" is a physical injury produced by force or shock. A traumatic injury is often associated with secondary complications, such as shock, respiratory failure and death.

For example, a traumatic injury can be caused by force of explosions or force of falling and flying objects. A traumatic injury also includes an injury to an internal organ resulting in hemorrhaging from the organ and/or at least partial loss of function. For example, an injury to an internal organ can be caused by penetration, such as from a bullet or flying projectile. A traumatic injury can also include injuries to musculoskeletal system, such as bones, muscles, cartilages, tendons, ligaments, joints and connective tissues. In one embodiment, a traumatic injury is a bone fracture.

Dermal injury is an injury to the dermis layer of the skin. Severe dermal injury is a dermal injury that results in dermal wound that covers at least 100 mm^2 and/or is full-thickness wound *(i.e.,* wound that penetrates through both the epidermis and dermis layer of the skin).

In one embodiment, the burn injury, traumatic injury or dermal injury sustained by the subject expose the subject to systemic infection. "Systemic infection" is an infection that has entered the blood stream and may affect multiple organs and/or tissues or the body as a whole.

In another embodiment, the thrombin peptide derivatives described herein reduce leucocytopenia and/or neutropenia in the subject being treated by the methods of the present invention. Alternatively, the thrombin peptide derivatives described herein reduces the decrease in population of bone marrow progenitor cells from radiation damage in the subject being treated by the methods of the present invention.

In another aspect, the present invention is a method of reducing the risk of developing systemic bacterial, fungal or viral infection in a subject, comprising administering to the subject an effective amount of a thrombin peptide derivative described herein. In one embodiment, the subject has been exposed to a bacterial, fungal or viral infection. In one example, a subject who

is at risk of developing systemic bacterial, viral or fungal infection has been exposed to radiation. For example, in the event of nuclear detonation, health workers, hospital patients, rescue workers, sanitarian workers and people who are in an environment or a place that is likely to have an outbreak of bacterial, fungal or viral infection, such as in a hospital. Alternatively, a subject who suffers from burn injury, traumatic injury or dermal injury resulting from radiation exposure, for example, from nuclear detonation, is more susceptible to bacterial, fungal or viral infection than a subject who does not suffer such injuries. In another alternative, a subject who has an unhealed wound prior to radiation exposure is more likely to develop bacterial, fungal or viral infection. In yet another alternative, a patient who is undergoing radiotherapy and has a pre-existing wound that is exposed to the radiation, for example from a surgery, would have higher risk for developing bacterial, fungal or viral infection than a patient who does not have a pre-existing wound. The thrombin peptide derivatives described herein are effective in reducing the risk of developing systemic bacterial, fungal or viral infection in the subjects described above upon radiation exposure.

In one embodiment, the method reduces the risk of developing bacterial, fungal or viral infection in the blood of the subject. High doses of radiation exposure can result in acute illness, such as breakdown of intestinal walls, which would render the subject more susceptible to septic infection. In one embodiment, the present invention is directed to a method of delaying the onset of septic systemic infection in a subject who is exposed to radiation comprising administering to the subject an effective amount of a thrombin peptide derivative described herein. The subject may be exposed to a lethal dose of radiation. Alternatively, the subject is exposed to a sub-lethal dose of radiation.

In another alternative, the subject is at risk of developing bacterial, fungal or viral

infection and has not been exposed to radiation. These subjects include one or more of the following: a) a subject has sustained traumatic injury, severe dermal injury and/or burn; b) a subject that underwent an invasive medical or dental procedure; c) a subject that underwent insertion of an invasive medical device; d) a subject who has pneumonia or other pulmonary conditions that could lead to acute respiratory distress syndrome or systemic infections; e) a subject who is immunocompromised; f) a subject who is an infant or is older than 60 years old, or g) subject from one or more of the categories a-d, who is an infant or older than 60 years old.

An invasive medical procedure that exposes a subject to infection is any procedure that involves either making a surgical cut in the skin or inserting an instrument, such as a needle or a tube, into the body of a subject. An invasive medical procedure increases a risk of introducing foreign organisms, such as bacteria or fungi, into the body of a subject, leading to an increased risk of bacterial, fungal or viral infection in the blood of the subject. An example of an invasive medical procedure is a surgery for any indication. Another example of an invasive medical procedure is a procedure wherein an invasive medical devise is introduced into a subject. Examples of invasive medical devises can include an intravenous or an arterial line, a breathing tube, a urinary catheter, a surgical drain, an artificial joint, or a feeding tube. Examples of feeding tubes can include G-tube/PEG tube, J-tube (jejunostomy tube) and NG-tube (nasogastric tube).

In another embodiment, the present invention is directed to a method of reducing the risk of developing a bacterial, fungal or viral infection in the blood of a subject who has pneumonia. Pneumonia is an inflammatory condition of the lung, especially of the alveoli. Infection is the most common cause of pneumonia. Infecting agents can be bacteria, viruses, fungi, or parasites. Chemical burns or physical injury to the lungs can also produce pneumonia. Bacteria are the

most common cause of pneumonia, with *Streptococcus pneumoniae* the most commonly isolated bacteria in the cases of community-acquired pneumonia. Another important Gram-positive cause of pneumonia is *Staphylococcus aureus*, with *Streptococcus agalactiae* being an important cause of pneumonia in newborn infants. Gram-negative bacteria cause pneumonia less frequently than gram-positive bacteria. Some of the gram-negative bacteria that cause pneumonia include *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Moraxella catarrhalis*. These bacteria often live in the stomach or intestines and may enter the lungs if vomit is inhaled. "Atypical" bacteria which cause pneumonia include *Chlamydophila pneumoniae*, *Mycoplasma pneumoniae*, and *Legionella pneumophila*.

In another embodiment, the present invention is directed to a method of reducing the risk of developing a bacterial, fungal or viral infection in the blood of a subject who is immunocompromised. An immunocompromised subject is a subject whose immune system is weakened or absent. Subjects who are immunocompromised are less capable of battling infections because of an immune response that is not properly functioning. Examples of immunocompromised subjects can include: a) subjects who have genetic defects that can affect functioning of their immune systems; b) subjects who have diseases such as AIDS or cancers, including leukemia, lymphoma or multiple myeloma; c) subjects who have chronic diseases, such as end-stage renal disease requiring dialysis, diabetes, or cirrhosis; d) subjects who receive treatments that can include steroids, chemotherapy, radiation, immunosuppressive post-transplant medications; and e) subjects who are pregnant.

In one embodiment, the bacterial, fungal or viral infection includes (in the presence or absence of radiation exposure), but is not limited to, infection of *staphylococci (e.g., staphylococcus aureus), enterococci, streptococci (e.g., streptococcus pneumoniae),*

pseudomonas aeruginosa, burkholderia cenocepacia, mycobacterium avium, enterobacter, bacteroides fragilis, streptococcus pyogenes, enterococcus sp., haemophilus influenzae, legionella sp., chlamydia pneumoniae, escherichia coli, clostridium sp., staphylococcus sp., *enterobacter* sp., *proteus* sp., *neiserria meningitidis , listeria monocytogenes, Candida* sp. *(e.g., Candida albicans), enterococcus* sp., *klebsiella, s. agalactiae,* and *aspergillus.* The bacterial, fungal or viral infection also includes systemic bacterial, systemic fungal infections and systemic viral infections.

In the event of nuclear detonation, people often suffer open dermal wounds in addition to radiation exposure. Additionally, radiation exposure can often cause skin injuries, such as skin ulceration. In the case of radiation therapy, cancer patients often undergo radiation therapy following surgical removal of the tumor and consequently have surgical wounds that are exposed to radiation when undergoing radiation therapy. A subject may also have a pre-existing wound before radiation exposure, including nuclear detonation, accidental radiation exposure or radiation therapy. The thrombin peptide derivatives described herein can promote healing of these wounds described above.

In one embodiment, the radiation exposure is sub-lethal. For example, the radiation exposure is less than 3.5 Gy when the subject is a human.

In yet another aspect, the present invention is directed to a method of reducing radiation related injury in a subject who is undergoing a radiation therapy. The method comprises administering to the subject an effective amount of a thrombin peptide derivative described herein.

A “radiation related injury” is an injury due to radiation exposure resulting from nuclear

detonation, nuclear weaponry, accidental radiation exposure or a radiation therapy. For example, when a subject is exposed to a high dose of radiation, such as in the event of nuclear detonation, the radiation exposure often causes acute illness in the subject, including hematopoietic syndrome as a result of effects of radiation on the bone marrow, spleen and lymph nodes, gastrointestinal syndrome (such as breakdown of the intestinal wall) due to the effects of radiation on the cells lining the digestive tract, and brain damage. Radiation exposure, such as radiation therapy, can also cause various skin injuries, such as intense reddening, blistering and ulceration of the skin at the irradiated site and late stage skin breakdown, and injury to hair follicles causing hair loss. Large dose of radiation exposure can cause permanent hair loss, damaged sebaceous and sweat glands, atrophy, fibrosis *(e.g.,* subcutaneous fibrosis), decreased or increased skin pigmentation, and ulceration or necrosis of the exposed tissue. In one embodiment, the radiation related injury is skin ulceration or late stage breakdown. Radiation exposure, such as radiation therapy, can also damage musculoskeletal system, such as bones, muscles, cartilages, tendons, ligaments, joints and connective tissues. In one embodiment, the present invention is directed to a method of promoting healing of a bone in a subject who is exposed and/or has been exposed to radiation exposure, wherein the bone is a fractured bone or has been surgically treated, for example, to remove tumor in the bone.

In one embodiment, for methods of reducing radiation related injury in a subject who is undergoing a radiation therapy, the thrombin peptide derivative is administered to normal tissue of the subject that is exposed or is to be exposed to the radiation. For example, the thrombin peptide derivative is topically administered to the normal skin that is exposed to the radiation. During a radiation therapy, radiation often causes damage to underlying tissues surrounding the target site of radiation, often because radiation exposure cannot be limited to the target site. To

reduce the radiation related injury to these normal tissues, the thrombin peptide derivative can be directly applied to the underlying tissues, locally *(e.g.,* by injection or implantation of a sustained release device and the like or through a catheter) or systemically, *e.g.,* before, during or after the radiation therapy. Alternatively, the thrombin peptide derivative can be applied or delivered locally or systemically during the radiation therapy or after the radiation therapy.

In yet another aspect, the present invention is directed to a method of reducing the risk of developing a radiation induced illness in a subject undergoing radiation therapy, comprising administering to the subject an effective amount of a thrombin peptide derivative described herein.

A "radiation induced illness" refers to a disorder, disease or condition that resulted from cellular damages caused by radiation exposure. For example, exposure to radiation can result in various cellular damages, such as damages to hematopeotic cells, decreased availability, viability and function of progenitor cells, delayed angiogensis and revascularization, apoptosis of intestinal microvascular endothelial cells, epithelial cells, crypt cells, neuronal cells in the brain and other tissues, and myocardium. As such, radiation induced illnesses include diseases, disorders or conditions resulting from the above-described cellular damages caused by radiation exposure. Exemplary radiation induced illness include, but is not limited to, leucocytopenia, neutropenia, infections, systemic inflammatory response syndrome (SIRS), sepsis, multiple organ dysfunction syndrome (MODS), lung damage, lung/airway disease, brain microvascular damage, brain cerebrovascular damage, stroke, atherosclerosis, peripheral vascular damage, peripheral artery disease (PAD), diabetic neuropathy and angiopathy and cancer.

In yet another aspect, the present invention is directed to a method of promoting healing

of a wound on a subject that was caused by radiation exposure and/or has been exposed to radiation. The method comprises administering to the wound (*e.g.*, topically or systemically, such as by I.V.) an effective amount of a thrombin peptide derivative described herein.

A wound is a type of injury in which skin is torn, cut or punctured (an open wound). An open wound can include incisions or incised wounds (caused by a clean, sharp-edged object such as a knife, a razor or a glass splinter); lacerations (irregular tear-like wounds caused by blunt trauma); abrasions; puncture wounds; penetration wounds or gunshot wounds (caused by a bullet or similar projectile driving into or through the body).

As used herein, the thrombin derivative peptides, the modified thrombin peptide derivatives and the thrombin peptide derivative dimers described below can be collectively referred to as "thrombin peptide derivatives." The thrombin derivative peptides, the modified thrombin peptide derivatives and each polypeptide in the thrombin peptide derivative dimers have 19 to 23 amino acids *(i.e.*, 19-23 amino acids in length).

Thrombin Derivative Peptides

Thrombin peptide derivatives (also: "thrombin derivative peptides") are analogs of thrombin that have an amino acid sequence derived at least in part from that of thrombin and are active at the non-proteolytically activated thrombin receptor (NPAR). Thrombin peptide derivatives can include, for example, peptides that are produced by recombinant DNA methods, peptides produced by enzymatic digestion of thrombin, and peptides produced synthetically, which can comprise amino acid substitutions compared to thrombin and/or modified amino

acids, especially at the termini.

Thrombin peptide derivatives of the present invention include thrombin derivative peptides described in U.S. Patent Nos. 5,352,664 and 5,500,412. In one embodiment, the thrombin peptide derivatives of the present invention is a thrombin peptide derivative or a physiologically functional equivalent, i.e., a polypeptide with no more than about fifty amino acids, preferably no more than about thirty amino acids and having sufficient homology to the fragment of human thrombin corresponding to thrombin amino acids 508-530 (Ala-Gly-Tyr-Lys-Pro-Asp-Glu-Gly-Lys-Arg-Gly-Asp-Ala-Cys-Glu-Gly-Asp-Ser-Gly-Gly-Pro-Phe-Val; SEQ ID NO:6) that the polypeptide activates NPAR.

In another embodiment, the thrombin peptide derivatives of the present invention is a thrombin peptide derivative comprising a moiety represented by Structural Formula (I):

Asp-Ala-R (I).

R is a serine esterase conserved domain. Serine esterases, e.g., trypsin, thrombin, chymotrypsin and the like, have a region that is highly conserved. "Serine esterase conserved domain" refers to a polypeptide having the amino acid sequence of one of these conserved regions or is sufficiently homologous to one of these conserved regions such that the thrombin peptide derivative retains NPAR activating ability.

A physiologically functional equivalent of a thrombin derivative encompasses molecules which differ from thrombin derivatives in aspects which do not affect the function of the thrombin receptor binding domain or the serine esterase conserved amino acid sequence. Such aspects may include, but are not limited to, conservative amino acid substitutions (as defined

below) and modifications, for example, amidation of the carboxyl terminus, acetylation of the amino terminus, conjugation of the polypeptide to a physiologically inert carrier molecule, or sequence alterations in accordance with the serine esterase conserved sequences.

A domain having a serine esterase conserved sequence can comprise a polypeptide sequence containing at least 4-12 of the N-terminal amino acids of the dodecapeptide previously shown to be highly conserved among serine proteases (Asp-X_1-Cys-X_2-Gly-Asp-Ser-Gly-Gly-Pro-X_3-Val; SEQ ID NO:13); wherein X_1, is either Ala or Ser; X_2 is either Glu or Gln; and X_3 is Phe, Met, Leu, His, or Val).

In one embodiment, the serine esterase conserved sequence comprises the amino acid sequence of SEQ ID NO:14 (Cys-Glu-Gly-Asp-Ser-Gly-Gly-Pro-Phe-Val) or a C-terminal truncated fragment of a polypeptide having the amino acid sequence of SEQ ID NO:14. It is understood, however, that zero, one, two or three amino acids in the serine esterase conserved sequence can differ from the corresponding amino acid in SEQ ID NO:14. Preferably, the amino acids in the serine esterase conserved sequence which differ from the corresponding amino acid in SEQ ID NO:14 are conservative substitutions as defined below, and are more preferably highly conservative substitutions. A "C-terminal truncated fragment" refers to a fragment remaining after removing an amino acid or block of amino acids from the C-terminus, said fragment having at least six and more preferably at least nine amino acids.

In another embodiment, the serine esterase conserved sequence comprises the amino acid sequence of SEQ ID NO:15 (Cys-X_1-Gly-Asp-Ser-Gly-Gly-Pro-X_2-Val; X_1 is Glu or Gln and X_2 is Phe, Met, Leu, His or Val) or a C-terminal truncated fragment thereof having at least six amino acids, preferably at least nine amino acids.

In a preferred embodiment, the thrombin peptide derivative comprises a serine esterase conserved sequence and a polypeptide having a more specific thrombin amino acid sequence Arg-Gly-Asp-Ala (SEQ ID NO:16). One example of a thrombin peptide derivative of this type comprises Arg-Gly-Asp-Ala-Cys-X_1-Gly-Asp-Ser-Gly-Gly-Pro-X_2-Val (SEQ ID NO: 1). X_1 and X_2 are as defined above. The thrombin peptide derivative can comprise the amino acid sequence of SEQ ID NO:6 (Ala-Gly-Tyr-Lys-Pro-Asp-Glu-Gly-Lys-Arg-Gly-Asp-Ala-Cys-Glu-Gly-Asp-Ser-Gly-Gly-Pro-Phe-Val) or an *N*-terminal truncated fragment thereof, provided that zero, one, two or three amino acids at positions 1-9 in the thrombin peptide derivative differ from the amino acid at the corresponding position of SEQ ID NO:6. Preferably, the amino acid residues in the thrombin peptide derivative which differ from the corresponding amino acid residues in SEQ ID NO:6 are conservative substitutions as defined below, and are more preferably highly conservative substitutions. An "*N*-terminal truncated fragment" refers to a fragment remaining after removing an amino acid or block of amino acids from the *N*-terminus, preferably a block of no more than six amino acids, more preferably a block of no more than three amino acids.

Optionally, the thrombin peptide derivatives described herein can be amidated at the C-terminus and/or acylated at the N-terminus. In a specific embodiment, the thrombin peptide derivatives comprise a C-terminal amide and optionally comprise an acylated N-terminus, wherein said C-terminal amide is represented by -C(O)NR_aR_b, wherein R_a and R_b are independently hydrogen, a C_1-C_{10} substituted or unsubstituted aliphatic group, or R_a and R_b, taken together with the nitrogen to which they are bonded, form a C1-C10 non-aromatic heterocyclic group, and said *N*-terminal acyl group is represented by $R_cC(0)$-, wherein R_L. is hydrogen, a C_1-C_{10} substituted or unsubstituted aromatic group, or a C_1-C_{10} substituted or unsubstituted aromatic group. In another specific embodiment, the *N*-terminus of the thrombin

peptide derivative is free (i.e., unsubstituted) and the *C*-terminus is free (i.e., unsubstituted) or amidated, preferably as a carboxamide (i.e., -C(O)NH_2). In a specific embodiment, the thrombin peptide derivative comprises the following amino acid sequence: Ala-Gly-Tyr-Lys-Pro-Asp-Glu-Gly-Lys-Arg-Gly-Asp-Ala-Cys-Glu-Gly-Asp-Ser-Gly-Gly-Pro-Phe-Val (SEQ ID NO:6). In another specific embodiment, the thrombin peptide derivative comprises the amino sequence of Arg-Gly-Asp-Ala-Cys-Glu-Gly-Asp-Ser-Gly-Gly-Pro-Phe-Val (SEQ ID NO:17). Alternatively, the thrombin peptide derivative comprises the amino acid sequence of SEQ ID NO:18: Asp-Asn-Met-Phe-Cys-Ala-Gly-Tyr-Lys-Pro-Asp-Glu-Gly-Lys-Arg-Gly-Asp-Ala-Cys-Glu-Gly-Asp-Ser-Gly-Gly-Pro-Phe-Val-Met-Lys-Ser-Pro-Phe. The thrombin peptide derivates comprising the amino acids of SEQ ID NO:6, 17, or 18 can optionally be amidated at the *C*-terminus and/or acylated at the *N*-terminus. Preferably, the *N*-terminus is free (i.e., unsubstituted) and the C-terminus is free (i.e., unsubstituted) or amidated, preferably a carboxamide (i.e., -C(O)NH_2). It is understood, however, that zero, one, two or three amino acids at positions 1-9 and 14-23 in the thrombin peptide derivative can differ from the corresponding amino acid in SEQ ID NO:6. It is also understood that zero, one, two or three amino acids at positions 1-14 and 19-33 in the thrombin peptide derivative can differ from the corresponding amino acid in SEQ ID NO:18. Preferably, the amino acids in the thrombin peptide derivative which differ from the corresponding amino acid in SEQ ID NO:6 or SEQ ID NO:18 are conservative substitutions as defined below, and are more preferably highly conservative substitutions. Alternatively, an *N*-terminal truncated fragment of the thrombin peptide derivative having at least fourteen amino acids or a *C*-terminal truncated fragment of the thrombin peptide derivative having at least eighteen amino acids can be used in the methods of the present invention.

A "*C*-terminal truncated fragment" refers to a fragment remaining after removing an

amino acid or block of amino acids from the *C*-terminus. An "*N*-terminal truncated fragment" refers to a fragment remaining after removing an amino acid or block of amino acids from the *N*-terminus. It is to be understood that the terms "*C*-terminal truncated fragment" and "*N*-terminal truncated fragment" encompass acylation at the *N*-terminus and/or amidation at the *C*-terminus, as described above.

A preferred thrombin peptide derivative for use in the disclosed method comprises the amino acid sequence SEQ ID NO:2: Ala-Gly-Tyr-Lys-Pro-Asp-Glu-GIy-Lys-Arg-Gly-Asp-Ala-Cys-X_1-Gly-Asp-Ser-Gly-Gly-Pro-X_2-Val. Another preferred thrombin peptide derivative for use in the disclosed method comprises the amino acid sequence of SEQ ID NO:19: Asp-Asn-Met-Phe-Cys-Ala-Gly-Tyr-Lys-Pro-Asp-Glu-Gly-Lys-Arg-Gly-Asp-Ala-Cys-X_1-Gly-Asp-Ser-Gly-Gly-Pro-X_2-Val-Met-Lys-Ser-Pro-Phe. X_1 is Glu or Gln; X_2 is Phe, Met, Leu, His or Val. The thrombin peptide derivatives of SEQ ID NO:2 and SEQ ID NO:19 can optionally comprise a C-terminal amide and/or acylated *N*-terminus, as defined above. Preferably, the *N*-terminus is free (i.e., unsubstituted) and the *C*-terminus is free (i.e., unsubstituted) or amidated, preferably as a carboxamide (i.e., -$C(O)NH_2$). Alternatively, N-terminal truncated fragments of these preferred thrombin peptide derivatives, the *N*-terminal truncated fragments having at least fourteen amino acids, or *C*-terminal truncated fragments of these preferred thrombin peptide derivatives, the *C*-terminal truncated fragments having at least eighteen amino acids, can also be used in the disclosed method.

TP508 is an example of a thrombin peptide derivative and is 23 amino acid residues long, wherein the *N*-terminal amino acid residue Ala is unsubstituted and the COOH of the *C*-terminal amino acid Val is modified to an amide represented by -$C(O)NH_2$ (SEQ ID NO:3). Another example of a thrombin peptide derivative comprises the amino acid sequence of SEQ ID NO:6,

wherein both *N*- and C-termini are unsubstituted ("deamide TP508"). Other examples of thrombin peptide derivatives which can be used in the disclosed method include *N*-terminal truncated fragments of TP508 (or deamide TP508), the N-terminal truncated fragments having at least fourteen amino acids, or C-terminal truncated fragments of TP508 (or deamide TP508), the *C*-terminal truncated fragments having at least eighteen amino acids.

As used herein, a "conservative substitution" in a polypeptide is the replacement of an amino acid with another amino acid that has the same net electronic charge and approximately the same size and shape. Amino acids with aliphatic or substituted aliphatic amino acid side chains have approximately the same size when the total number of carbon and heteroatoms in their side chains differs by no more than about four. They have approximately the same shape when the number of branches in their side chains differs by no more than one. Amino acids with phenyl or substituted phenyl groups in their side chains are considered to have about the same size and shape. Listed below are five groups of amino acids. Replacing an amino acid in a polypeptide with another amino acid from the same group results in a conservative substitution:

Group I: glycine, alanine, valine, leucine, isoleucine, serine, threonine, cysteine, and non-naturally occurring amino acids with C1-C4 aliphatic or C1-C4 hydroxyl substituted aliphatic side chains (straight chained or monobranched).

Group II: glutamic acid, aspartic acid and non-naturally occurring amino acids with carboxylic acid substituted C1-C4 aliphatic side chains (unbranched or one branch point).

Group III: lysine, ornithine, arginine and non-naturally occurring amino acids with amine or guanidino substituted C1-C4 aliphatic side chains (unbranched or one branch point).

Group IV: glutamine, asparagine and non-naturally occurring amino acids with amide substituted Cl -C4 aliphatic side chains (unbranched or one branch point).

Group V: phenylalanine, phenylglycine, tyrosine and tryptophan.

As used herein, a "highly conservative substitution" in a polypeptide is the replacement of an amino acid with another amino acid that has the same functional group in the side chain and nearly the same size and shape. Amino acids with aliphatic or substituted aliphatic amino acid side chains have nearly the same size when the total number of carbon and heteroatoms in their side chains differs by no more than two. They have nearly the same shape when they have the same number of branches in the their side chains. Examples of highly conservative substitutions include valine for leucine, threonine for serine, aspartic acid for glutamic acid and phenylglycine for phenylalanine. Examples of substitutions which are not highly conservative include alanine for valine, alanine for serine and aspartic acid for serine.

Modified Thrombin Peptide Derivatives

In one embodiment of the invention, the thrombin peptide derivatives are modified relative to the thrombin peptide derivatives described above, wherein cysteine residues of aforementioned thrombin peptide derivatives are replaced with amino acids having similar size and charge properties to minimize dimerization of the peptides. Examples of suitable amino acids include alanine, glycine, serine, or an S'-protected cysteine. Preferably, cysteine is replaced with alanine. The modified thrombin peptide derivatives have about the same biological activity as the unmodified thrombin peptide derivatives. See Publication No. US 2005/0158301 Al,

which is hereby incorporated by reference.

It will be understood that the modified thrombin peptide derivatives disclosed herein can optionally comprise C-terminal amides and/or N-terminal acyl groups, as described above. Preferably, the *N*-terminus of a thrombin peptide derivative is free (i.e., unsubstituted) and the *C*-terminus is free (i.e., unsubstituted) or amidated, preferably as a carboxamide (i.e., -C(O)NH_2).

In a specific embodiment, the modified thrombin peptide derivative comprises a polypeptide having the amino acid sequence of SEQ ID NO:4: Arg-Gly-Asp-Ala-Xaa-X_1-Gly-Asp-Ser-Gly-Gly-Pro-X_2-Val, or a C-terminal truncated fragment thereof having at least six amino acids. More specifically, the thrombin peptide derivative comprises the amino acid sequence of SEQ ID NO:20: Ala-Gly-Tyr-Lys-Pro-Asp-Glu-Gly-Lys-Arg-Gly-Asp-Ala-Xaa-Glu-Gly-Asp-Ser-Gly-Gly-Pro-Phe-Val or a fragment thereof comprising amino acids 10-18 of SEQ ID NO:20. Even more specifically, the thrombin peptide derivative comprises the amino acid sequence SEQ ID NO:5: Ala-Gly-Tyr-Lys-Pro-Asp-Glu-Gly-Lys-Arg-Gly-Asp-Ala-Xaa-X_1-Gly-Asp-Ser-Gly-Gly-Pro-X_2-Val, or a fragment thereof comprising amino acids 10-18 of SEQ ID NO:5. Xaa is alanine, glycine, serine or an S-protected cysteine. X_1 is Glu or Gln and X_2 is Phe, Met, Leu, His or Val. Preferably X_1 is Glu, X_2 is Phe, and Xaa is alanine. One example of a thrombin peptide derivative of this type is a polypeptide having the amino acid sequence Ala-Gly-Tyr-Lys-Pro-Asp-Glu-Gly-Lys-Arg-Gly-Asp-Ala-Ala-Glu-Gly-Asp-Ser-Gly-Gly-Pro-Phe-Val (SEQ ID NO:21). A further example of a thrombin peptide derivative of this type is the polypeptide H-Ala-Gly-Tyr-Lys-Pro-Asp-Glu-Gly-Lys-Arg-Gly-Asp-Ala-Ala-Glu-Gly-Asp-Ser-Gly-Gly-Pro-Phe-Val-NH_2 (SEQ ID NO:22). Another example of a thrombin peptide derivative of this type is the polypeptide H-Ala-Gly-Tyr-Lys-Pro-Asp-Glu-Gly-Lys-Arg-Gly-Asp-Ala-Ser-Glu-Gly-Asp-Ser-Gly-Gly-Pro-Phe-Val-NH_2 (SEQ ID NO:30) Zero, one, two

or three amino acids in the thrombin peptide derivative differ from the amino acid at the corresponding position of SEQ ID NO:4, 20, 5, 21 or 22, provided that Xaa is alanine, glycine, serine or an *S*-protected cysteine. Preferably, the difference is conservative as defined below.

In another specific embodiment, the thrombin peptide derivative comprises a polypeptide having the amino acid sequence SEQ ID NO:23: Asp-Asn-Met-Phe-Xbb-Ala-Gly-Tyr-Lys-Pro-Asp-Glu-Gly-Lys-Arg-Gly-Asp-Ala-Xaa-Glu-Gly-Asp-Ser-Gly-Gly-Pro-Phe-Val-Met-Lys-Ser-Pro-Phe, or a fragment thereof comprising amino acids 6-28. More preferably, the thrombin peptide derivative comprises a polypeptide having the amino acid sequence SEQ ID NO:24: Asp-Asn-Met-Phe-Xbb-Ala-Gly-Tyr-Lys-Pro-Asp-Glu-Gly-Lys-Arg-Gly-Asp-Ala-Xaa-X_1-Gly-Asp-Ser-Gly-Gly-Pro-X_2-Val-Met-Lys-Ser-Pro-Phe, or a fragment thereof comprising amino acids 6-28. Xaa and Xbb are independently alanine, glycine, serine or an S-protected cysteine. X_1 is Glu or Gln and X_2 is Phe, Met, Leu, His or Val. Preferably X_1 is Glu, X_2 is Phe, and Xaa and Xbb are alanine. One example of a thrombin peptide derivative of this type is a polypeptide comprising the amino acid sequence Asp-Asn-Met-Phe-Ala-Ala-Gly-Tyr-Lys-Pro-Asp-Glu-Gly-Lys-Arg-Gly-Asp-Ala-AIa-Glu-Gly-Asp-Ser-Gly-Gly-Pro-Phe-Val-Met-Lys-Ser-Pro-Phe(SEQ ID NO:25). A further example of a thrombin peptide derivative of this type is the polypeptide H-Asp-Asn-Met-Phe-Ala-Ala-Gly-Tyr-Lys-Pro-Asp-Glu-Gly-Lys-Arg-Gly-Asp-Ala-Ala-Glu-Gly-Asp-Ser-Gly-Gly-Pro-Phe-Val-Met-Lys-Ser-Pro-Phe-NH_2 (SEQ ID NO:26). Zero, one, two or three amino acids in the thrombin peptide derivative can differ from the amino acid at the corresponding position of SEQ ID NO:23, 24, 25 or 26. Xaa and Xbb are independently alanine, glycine, serine or an S-protected cysteine. Preferably, the difference is conservative as in conservative substitutions of the thrombin peptide derivatives.

An "*S*-protected cysteine" is a cysteine residue in which the reactivity of the thiol moiety,

-SH, is blocked with a protecting group. Suitable protecting groups are known in the art and arc disclosed, for example, in T. W. Greene and P. G. M. Wuts, *Protective Groups in Organic Synthesis,* 3rd Edition, John Wiley & Sons, (1999), pp. 454-493, the teachings of which are incorporated herein by reference in their entirety. Suitable protecting groups should be non-toxic, stable in pharmaceutical formulations and have minimum additional functionality to maintain the activity of the thrombin peptide derivative. A free thiol can be protected as a thioether, a thioester, or can be oxidized to an unsymmetrical disulfide. Preferably the thiol is protected as a thioether. Suitable thioethers include, but are not limited to, S-alkyl thioethers (e.g., C_1-C_5 alkyl), and S-benzyl thioethers (e.g, cysteine-S-S-*t*-Bu). Preferably the protective group is an alkyl thioether. More preferably, the S-protected cysteine is an S-methyl cysteine. Alternatively, the protecting group can be: 1) a cysteine or a cysteine-containing peptide (the "protecting peptide") attached to the cysteine thiol group of the thrombin peptide derivative by a disulfide bond; or 2) an amino acid or peptide ("protecting peptide") attached by a thioamide bond between the cysteine thiol group of the thrombin peptide derivative and a carboxylic acid in the protecting peptide (e.g., at the C-terminus or side chain of aspartic acid or glutamic acid). The protecting peptide can be physiologically inert (e.g., a polyglycine or polyalanine of no more than about fifty amino acids optionally interrupted by a cysteine), or can have a desirable biological activity.

Thrombin Peptide Derivative Dimers

In some aspects of the present invention, the thrombin peptide derivatives of the methods are thrombin peptide derivative dimers. See Publication No. US 2005/0153893, which is hereby incorporated by reference. The dimers essentially do not revert to monomers and still have about the same biological activity as the thrombin peptide derivatives monomer described above. A

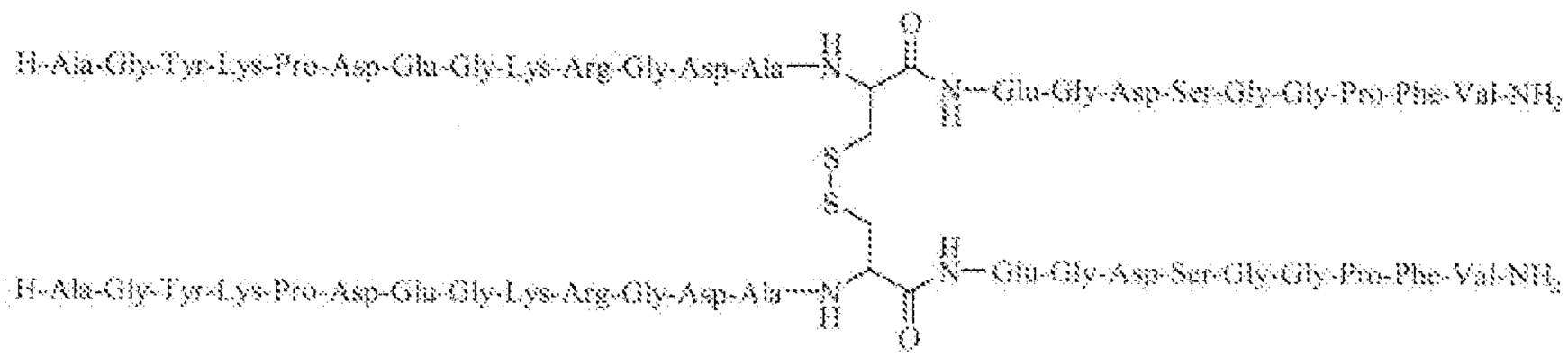
"thrombin peptide derivative dimer" is a molecule comprising two thrombin peptide derivatives linked by a covalent bond, preferably a disulfide bond between cysteine residues. Thrombin peptide derivative dimers are typically essentially free of the corresponding monomer, e.g., greater than 95% free by weight and preferably greater than 99% free by weight. Preferably the polypeptides are the same and covalently linked through a disulfide bond.

The thrombin peptide derivative dimers of the present invention comprises the thrombin peptide derivatives described above. Specifically, thrombin peptide derivatives have less than about fifty amino acids, preferably less than about thirty-three amino acids. Thrombin peptide derivatives also have sufficient homology to the fragment of human thrombin corresponding to thrombin amino acid residues 508-530: Ala-Gly-Tyr-Lys-Pro-Asp-Glu-Gly-Lys-Arg-Gly-Asp-Ala-Cys-Glu-Gly-Asp-Ser-Gly-Gly-Pro-Phe-Val (SEQ ID NO:6) so that the polypeptide activates NPAR.

In a specific embodiment, each thrombin peptide derivative comprising a dimer comprises a polypeptide having the amino acid sequence SEQ ID NO:1: Arg-Gly-Asp-Ala-Cys-X_1-Gly-Asp-Ser-Gly-Gly-Pro-X_2-Val, or a *C*-terminal truncated fragment thereof comprising at least six amino acids. More specifically, each thrombin peptide derivative comprises the amino acid sequence of SEQ ID NO:6: Ala-Gly-Tyr-Lys-Pro-Asp-Glu-Gly-Lys-Arg-Gly-Asp-Ala-Cys-Glu-Gly-Asp-Ser-Gly-Gly-Pro-Phe-Val, or a fragment thereof comprising amino acids 10-18 of SEQ ID NO. 5. Even more specifically, the thrombin peptide derivative comprises the amino acid sequence SEQ ID NO:2: Ala-Gly-Tyr-Lys-Pro-Asp-Glu-Gly-Lys-Arg-Gly-Asp-Ala-Cys-X_1-Gly-Asp-Ser-Gly-Gly-Pro-X_2-Val, or a fragment thereof comprising amino acids 10-18 of SEQ ID NO:2. X_1 is Glu or Gln and X_2 is Phe, Met, Leu, His or Val. Preferably X_1 is Glu, and X_2 is Phe. One example of a thrombin peptide derivative of this type is a polypeptide comprising

the amino acid sequence Ala-Gly-Tyr-Lys-Pro-Asp-Glu-Gly-Lys-Arg-Gly-Asp-Ala-Cys-Glu-Gly-Asp-Ser-Gly-Gly-Pro-Phe-Val (SEQ ID NO:6). A further example of a thrombin peptide derivative of this type is a polypeptide having the amino acid sequence H-Ala-Gly-Tyr-Lys-Pro-Asp-Glu-Gly-Lys-Arg-Gly-Asp-Ala-Cys-Glu-Gly-Asp-Ser-Gly-Gly-Pro-Phe-Val-NH_2 (SEQ ID NO:3). Zero, one, two or three amino acids in the thrombin peptide derivative differ from the amino acid at the corresponding position of SEQ ID NO:6, 1, 2, or 3. Preferably, the difference is conservative as for conservative substitutions of the thrombin peptide derivatives.

One example of a thrombin peptide derivative dimer of the present invention is represented by Formula (IV):



(IV).

In another specific embodiment, each thrombin peptide derivative comprising a dimer comprises a polypeptide comprising the amino acid sequence SEQ ID NO:27: Ala-Gly-Tyr-Lys-Pro-Asp-Glu-Gly-Lys-Arg-Gly-Asp-Ala-Cys-Glu-Gly-Asp-Ser-Gly-Gly-Pro-Phe-Val-Met-Lys-Ser-Pro-Phe-Asn-Asn-Arg-Trp-Tyr, or a *C*-terminal truncated fragment thereof having at least twenty-three amino acids. More preferably, each thrombin peptide derivative comprises the amino acid sequence SEQ ID NO:28: Ala-Gly-Tyr-Lys-Pro-Asp-Glu-Gly-Lys-Arg-Gly-Asp-Ala-Cys-X_1-Gly-Asp-Ser-Gly-Gly-Pro-X_2-Val-Met-Lys-Ser-Pro-Phe-Asn-Asn-Arg-Trp-Tyr, or a C-terminal truncated fragment thereof comprising at least twenty-three amino acids. Xi is Glu

or Gln and X_2 is Phe, Met, Leu, His or Val. Preferably X_1 is Glu, and X_2 is Phe. One example of a thrombin peptide derivative of this type is a polypeptide comprising the amino acid sequence Ala-Gly-Tyr-Lys-Pro-Asp-Glu-Gly-Lys-Arg-Gly-Asp-Ala-Cys-Glu-Gly-Asp-Ser-Gly-Gly-Pro-Phe-Val-Met-Lys-Ser-Pro-Phe-Asn-Asn-Arg-Trp-Tyr (SEQ ID NO:27). A further example of a thrombin peptide derivative of this type is a polypeptide comprising the amino acid sequence H-Ala-Gly-Tyr-Lys-Pro-Asp-Glu-Gly-Lys-Arg-Gly-Asp-Ala-Cys-Glu-Gly-Asp-Ser-Gly-Gly-Pro-Phe-Val-Met-Lys-Ser-Pro-Phe-Asn-Asn-Arg-Trp-Tyr-NH_2 (SEQ ID NO:29). Zero, one, two or three amino acids in the thrombin peptide derivative differ from the amino acid at the corresponding position of SEQ ID NO:27, 28 or 29. Preferably, the difference is conservative as defined for conservative substitutions of the thrombin peptide derivatives.

A "subject" is preferably a human, but can also be an animal in need of treatment with a thrombin peptide derivative disclosed herein, e.g., companion animals (e.g., dogs, cats, and the like), farm animals (e.g., cows, pigs, horses and the like) and laboratory animals (e.g., rats, mice, guinea pigs and the like).

An "effective amount" is the quantity of the thrombin peptide derivative described herein that results in an improved clinical outcome of the condition being treated with the thrombin peptide derivative compared with the absence of treatment. The amount of the thrombin peptide derivative administered will depend on the degree, severity, and type of the disease or condition, the amount of therapy desired, and the release characteristics of the pharmaceutical formulation. It will also depend on the subject's health, size, weight, age, sex and tolerance to drugs. Typically, the thrombin peptide derivative is administered for a sufficient period of time to achieve the desired therapeutic effect. Typically, from about 1 μg per day to about 1 mg per day of the thrombin peptide derivatives (preferably from about 5 μg per day to about 100 μg per day)

is administered to the subject in need of treatment, especially for a local means of administration. The thrombin peptide derivatives can also be administered at a dose of from about 0.1 mg/kg/day to about 15 mg/kg/day, with from about 0.2 mg/kg/day to about 3 mg/kg/day being preferred, especially for systemic means of administration. Typical dosages for the thrombin peptide derivative of the invention are also 5-500 mg/day, preferably 25-250 mg/day, especially for systemic means of administration.

In the methods described herein, the thrombin peptide derivative or composition can be administered before, during or after the radiation exposure. In the methods described herein, the peptide of the present invention can be administered in combination with an angiogenic growth factor. An "angiogenic growth factor" is a polypeptide which stimulates the development of blood vessels, e.g., promotes angiogenesis, endothelial cell growth, stability of blood vessels, and/or vasculogenesis. For example, angiogenic factors, include, but are not limited to, e.g., VEGF-A and members of the VEGF family, PlGF, PDGF family, fibroblast growth factor family (FGFs), TIE ligands (Angiopoietins), ephrins, ANGPTL3, ANGPTL4, etc. Angiogenic factors also include polypeptides, such as growth hormone, insulin-like growth factor-I (IGF-I), VIGF, epidermal growth factor (EGF), CTGF and members of its family, and TGF-α and TGF-β.

"Treating" means that following a period of administering the thrombin peptide derivative or composition comprising a thrombin peptide derivative, a beneficial therapeutic and/or prophylactic result is achieved, which can include a decrease in the severity of symptoms or delay in or inhibition of the onset of symptoms, increased longevity and/or more rapid or more complete resolution of the disease or condition, or other improved clinical outcome as measured according to the site that is being observed or the parameters measured for a particular disease or disorder.

"Reducing the risk" refers to decreasing the probability of developing a disease, disorder or medical condition, in a subject, wherein the subject is, for example, a subject who is at risk for developing the disease, disorder or condition.

"Reducing radiation related injury" refers to a decrease in the severity of injuries induced by radiation exposure.

The disclosed thrombin peptide derivative can be administered by any suitable route, locally *(e.g.,* topically) or systemically, including, for example, by parenteral administration. Parenteral administration can include, for example, intramuscular, intravenous, subcutaneous, or intraperitoneal injection or vascular administration, and can also include transdermal patch and implanted slow-release devices such as pumps. Topical administration can include, for example, creams, gels, ointments or aerosols. Respiratory administration can include, for example, inhalation or intranasal drops. For certain indications, it is advantageous to inject or implant the thrombin peptide derivative directly to the treatment site. The thrombin peptide derivative can be advantageously administered in a sustained release formulation. The thrombin peptide derivative can be administered chronically, wherein the peptide derivative is administered over a long period of time (at least 60 days, but more typically, for at least one year), at intervals or by a continuous delivery method, to treat a chronic or recurring disease or condition.

The thrombin peptide derivative can be administered to the subject in conjunction with an acceptable pharmaceutical carrier as part of a pharmaceutical composition. The formulation of the pharmaceutical composition will vary according to the route of administration selected. Suitable pharmaceutical carriers may contain inert ingredients which do not interact with the compound. The earners should be biocompatible, i.e., non-toxic, non-inflammatory, non-

immunogenic and devoid of other undesired reactions at the administration site. Examples of pharmaceutically acceptable carriers include, for example, saline, aerosols, commercially available inert gels, or liquids supplemented with albumin, methyl cellulose or a collagen matrix. Standard pharmaceutical formulation techniques can be employed, such as those described in Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, PA. Other suitable pharmaceutical carriers include those described in U.S. Patent No. 7,294,596, the entire teaching of which is incorporated herein by reference.

The compositions used in the methods of the present invention can additionally comprise a pharmaceutical carrier in which the thrombin peptide derivative is dissolved or suspended. Examples of pharmaceutically acceptable carriers include, for example, saline, aerosols, commercially available inert gels, or liquids supplemented with albumin, methyl cellulose or a collagen matrix. Typical of such formulations are gels. Gels are comprised of a base selected from an oleaginous base, water, or an emulsion-suspension base, as previously described. To the base is added a gelling agent that forms a matrix in the base, increasing its viscosity to a semisolid consistency. Examples of gelling agents are hydroxypropyl cellulose, acrylic acid polymers, and the like. The active ingredients are added to the formulation at the desired concentration at a point preceding addition of the gelling agent or can be mixed after the gelation process.

Injectable delivery formulations may be administered intravenously or directly at the site in need of treatment. The injectable carrier may be a viscous solution or gel.

Delivery formulations include physiological saline, bacteriostatic saline (saline containing about 0.9% mg/mL benzyl alcohol), phosphate-buffered saline, Hank's solution,

Ringer's-lactate, or liquids supplemented with albumin, methyl cellulose, or hyaluronic acid. Injectable matrices include polymers of poly(ethylene oxide) and copolymers of ethylene and propylene oxide (see Cao *et al, J. Biomater. Sci* 9:475 (1998) and Sims *et al, Plast Reconstr. Surg. 98:843* (1996), the entire teachings of which are incorporated herein by reference).

Methods for encapsulating compositions (such as in a coating of hard gelatin or cyclodextran) are known in the art (Baker, *et al,* "Controlled Release of Biological Active Agents", John Wiley and Sons, 1986).

Ointments are typically prepared using an oleaginous base, e.g., containing fixed oils or hydrocarbons, such as white petrolatum or mineral oil, or an absorbent base, e.g., consisting of an absorbent anhydrous substance or substances, for example anhydrous lanolin. Following formation of the base, the active ingredients are added in the desired concentration.

Creams generally comprise an oil phase (internal phase) containing typically fixed oils, hydrocarbons, and the like, such as waxes, petrolatum, mineral oil, and the like, and an aqueous phase (continuous phase), comprising water and any water-soluble substances, such as added salts. The two phases are stabilized by use of an emulsifying agent, for example, a surface active agent, such as sodium lauryi sulfate; hydrophilic colloids, such as acacia colloidal clays, beegum, and the like. Upon formation of the emulsion, the active ingredients are added in the desired concentration.

Gels contain a base selected from an oleaginous base, water, or an emulsion-suspension base, as previously described. To the base is added a gelling agent which forms a matrix in the base, increasing its viscosity to a semisolid consistency. Examples of gelling agents are hydroxypropyl cellulose, acrylic acid polymers, and the like. The active ingredients are added to

the formulation at the desired concentration at a point preceding addition of the gelling agent.

Diseases and conditions that are treatable with the disclosed thrombin peptide derivatives are often accompanied by symptoms and infirmities such as pain and infection. In certain instances it may be advantageous to co-administer one or more additional pharmacologically active agents along with a thrombin peptide derivative to address such issues. For example, managing pain and inflammation may require co-administration with analgesic or an anti-inflammatory agents. Managing infection may require co-administration with antimicrobial, antibiotic or disinfectant agents.

A thrombin peptide derivative can be administered to a subject alone or in combination with one or more other therapeutics, for example, a cholesterol-lowering agent, an anti-hypertensive agent, a beta-blocker, an anti-coagulant, a thrombolytic agent, an analgesic, an anti-inflammatory agent, an anti-plaque agent, insulin, a nitric oxide generating agent, an antiviral agent or an antibiotic. In one method, a thrombin peptide derivative can be administered to a subject in combination with arginine, for example, with arginine as an oral nutritional supplement.

Thrombin peptide derivatives and modified thrombin peptide derivatives can be synthesized by solid phase peptide synthesis (e.g., BOC or FMOC) method, by solution phase synthesis, or by other suitable techniques including combinations of the foregoing methods. The BOC and FMOC methods, which are established and widely used, are described in Merrifield, *J. Am. Chem. Sot: 88:2149* (1963); Meienhofer, *Hormonal Proteins and Peptides,* C.H. Li, Ed., Academic Press, 1983, pp. 48-267; and Barany and Merrifield, in *The Peptides,* E. Gross and J. Meienhofer, Eds., Academic Press, New York, 1980, pp. 3-285. Methods of solid phase peptide

synthesis are described in Merrifield, R.B., *Science, 232:* 341 (1986); Carpino, L.A. and Han, G.Y., *J. Org. Chem., 37:* 3404 (1972); and Gauspohl, H. *et al, Synthesis,* J: 315 (1992)). The teachings of these six articles are incorporated herein by reference in their entirety.

Thrombin peptide derivative dimers can be prepared by oxidation of the monomer. Thrombin peptide derivative dimers can be prepared by reacting the thrombin peptide derivative with an excess of oxidizing agent. A well-known suitable oxidizing agent is iodine.

A "non-aromatic heterocyclic group" as used herein, is a non-aromatic carbocyclic ring system that has 3 to 10 atoms and includes at least one heteroatom, such as nitrogen, oxygen, or sulfur. Examples of non-aromatic heterocyclic groups include piperazinyl, piperidinyl, pyrrolidinyl, morpholinyl, thiomorpholinyl.

An "alkyl" is a straight chain or branched saturated hydrocarbon radical. Typically, an alkyl group has from 1 to about 10 carbon atoms, preferably from 1 to about 4 carbon atoms. Exemplary alkyl groups include, but are not limited to, methyl, ethyl, n-propyl, iso-propyl, n-butyl, sec-butyl, tert-butyl, pentyl, cyclopentyl, hexyl, cyclohexyl, octyl and cyclooctyl.

The invention is illustrated by the following examples which are not intended to be limiting in any way.

Example 1. Effects of TP508 on apoptosis of human microvasular endothelial cell (HMVEC) exposed to radiation

Human dermal microvascular endothelial cells (HMVEC) were irradiated using a J.L., Shepherd & Associates, Mark 1, 9K, ^{137}Cs Gamma Irradiator to deliver exposures of 5, 10, and 20 Gy. Following irradiation, cells received 30 u-g/ml TP508 or saline (No Treatment). After 3h

cells were assayed for activation of caspase-3 and caspase-7 as a measure of apoptosis using Caspase-Glo® 3/7 Assay (Promega, Madison, WI). As shown in Figure 1, radiation caused a dose-dependent increase in Caspase 3/7 activity. TP508 treatment of these cells, however, significantly decreased radiation-induced activation of Caspase 3/7. This data demonstrates that TP508 attenuates apoptosis in microvascular endothelial cells induced by radiation.

Example 2. Effect of TP508 on mouse survival following 8 Gy radiation exposure.

Swiss ICR mice were irradiated (^{137}Cs Gamma Irradiator Mark 30, Shephard and Associates, San Fernando, CA) with exposures of 8 Gy or 3 Gy. After 4 hours or 24 hours, mice were anesthesized and prepared for surgery. A single 1.5 cm square full dermal excision was created and treated topically with saline (25 µl) or saline plus TP508 (0.25 µg) and covered with Opsite® occlusive dressing. There was a significant decrease in survival in mice that have sustained radiation combined injuries (Table 1 below). Significant increase in survival was observed when mice were injected with TP508 24 hours after radiation exposure (see Table 1). Increase in survival was also observed in mice receiving topical treatment of wounds with TP508 (Table 1 and Figure 2).

Table 1. Effect of TP508 on Survival of Mice with Radiation Combined Injuries

Group	Treatment	Mean Survival (days)	St. Dev.	Median Survival (days)	30-day survival (%)	Significance
0 Gy + Wound	Saline	30.0	0	Undefined >30	100	1

8 Gy + Wound	Saline	13.4	6.0	11	10	2,3
8 Gy + Wound	TP508 Topical	19.1	9.1	14	37	3
8 Gy + Wound	TP508 I.V.	25.2	7.5	Undefined >30	67	2
8 Gy	none	21.3	9.3	15	40	1

1. Significant decrease in survival when radiation is combined with wounds ($p = 0.0334$)
2. Significant increase in survival when mice injected with TP508 24 hr after radiation exposure ($p = 0.0014$)
3. Increase in survival after treatment of wounds topically (NS, $p = 0.1406$))

Example 3. Effect of TP508 on mouse survival following radiation exposure to the lethal dose of 12 Gy.

Mice were exposed to a lethal dose of ^{137}Cs gamma irradiation (12 Gy). Injection of a single bolus dose of TP508 (500 μg) within 2 hours after exposure delayed the mortality of the first mouse in the treated group by about 3 days and increased the group mean survival time by about 15%. (See Figure 3). TP508 has a short half-life and may thus only be present in blood at an effective concentration for the first two to three hours. This may explain why it only extends survival for a few days.

Example 4. Effect of TP508 on bacterial growth in blood of animals post irradiation

Lethal doses of radiation often cause death due to breakdown of the intestinal wall and septic infection leading to death. Therefore, the effect of TP508 to delay the onset of bacterial

septic infection in irradiated mice was determined. Blood was drawn from irradiated mice (see Example 3) at various days after irradiation. Blood from each mouse (3 mice per group) was then diluted and cultured to determine the number of live bacteria quantified as colony forming units (CPU) per ml of blood. As shown in Figure 4, by day 6 post irradiation (PI), live bacteria were present in the blood of irradiated placebo-treated mice, but not from TP508-treated mice. By day 7 the placebo-treated mice had an average of 1.6×10^6 CFU/ml while those injected with TP508 were just beginning to show infection, with an average of just over 100 CFU/ml.

Example 5. Effect of TP508 on healing of open dermal wounds

Swiss ICR mice were irradiated (^{137}Cs Gamma Irradiator Mark 30, Shephard and Associates, San Fernando, CA) with exposures of 8 Gy or 3 Gy. After 4 hours or 24 hours, mice were anesthesized and prepared for surgery. A single 1.5 cm square full dermal excision was created and treated topically with saline (25 ul) or saline plus TP508 (0.3 ug) and covered with Opsite® occlusive dressing. At 8 Gy, radiation delayed wound healing in mice receiving dermal wounds 4 hours after irradiation, but a single topical treatment with TP508 accelerated healing. The time to 50% wound closure of these wounds was 9.2 days for non-irradiated control; 13.0 days for 8 GY plus saline; and 8.9 days for 8 Gy plus TP508. Thus, TP508 appears to restore normal rates of healing to irradiated mice. This was confirmed by calculating the linear rate of healing in these wounds (See Figure 5). Interestingly, the linear rates of healing for all groups was similar during the first 5 days after wounding, perhaps due to contraction that was not affected by radiation. From 5 to 16 days, however, radiation significantly impairs healing, but TP508 treatment overcomes this impairment.

In a second set of experiments, mice with 3 Gy exposures underwent dermal wound

surgery 24 hours after irradiation. These wounds also demonstrated delayed healing relative to non-irradiated control mice. As with 8 Gy exposure experiments, TP508 topical treatment accelerated healing to overcome the effect of radiation. In these experiments, we also evaluated effects of post-exposure IV injection of TP508 on wound closure. An IV injection of TP508 about 20 hours prior to wound injury also accelerated wound closure and tended to close wounds slightly faster than topical treatment. This slight difference is also seen in comparisons of the rates of linear wound healing between non-irradiated control, 3 Gy Saline Control, 3 Gy topical TP508 and 4 Gy IV TP508. The combination of IV and topical TP508 treatment did not appear to be different than IV treatment alone.

Example 6. Effect of TP508 on apoptosis and proliferation and migration of intestinal crypt progenitor cells.

At 5 days post-exposure, histological sections of jujenum taken from mice exposed to 12 Gy whole body irradiation contain a large number of apoptotic cells within the intestinal cryps, as determined by tunnel staining. Mice injected with TP508 appear to have fewer apoptotic cells. This effect of TP508 was confirmed by measuring EdU incorporation (DNA synthesis) at 2 and 12 days post-exposure, visualizing cells that synthesized DNA during a 24-hour incubation period with Click IT®. With increasing radiation exposure fewer crypt cells continue to proliferate after 2 days. In contrast, with TP508 injection the number of cells proliferating and migrating out of the crypt with 3 Gy exposure is equivalent to non-irradiated (0 Gy) controls. In the 8 Gy sections, approximately the same number of cells are labeled, but in the TP508 group, cells tend to migrate farther up into the villi. Even after 15 Gy exposures, some crypt cells continue to proliferate in cryps of animals treated with TP508. Even 12 days after 8 Gy exposures there is decreased crypt cells proliferation and migration, yet in animals injected with

TP508, the proliferation and migration appears to be fully restored to control levels.

Example 7. Effect of TP508 on radiation and RCI-induced up-regulation of IL-6.

ICR white male mice were exposed to 0 Gy (control) or 8 Gy of gamma radiation and wounded 24 hours later. Wounds were treated topically with saline placebo (P) or TP508 in saline (TPt). A separate group of mice were injected IV with 500 micrograms of TP508 2 hours post 8 Gy exposure. After eleven (11) days, serum from mice was analyzed for amount of IL-6A using enzyme-linked immunosorbent assay (ELISA) (Figure 6, Panel A).

ICR white male mice were exposed to 0 Gy, or 12 Gy nuclear irradiaton without wounds and were injected IV with placebo or TP508 post-exposure. Serum was isolated from mice seven (7) days later and the amount of IL-6 was determined by enzyme-linked immunosorbent assay (ELISA) (Figure 6, Panel B).

The combination of 8Gy radiation exposure and wounding increases IL-6 levels above wounding alone. Topical TP508 treatment of wounds reduces IL-6 levels by ~75%. Systemic IV injection of TP508 reduces IL-6 levels by more than 90%. 12Gy exposure alone without wounds also increases IL-6 levels. TP508 injection reduces IL-6 production measured at day 7 by approximately 50%.

Since IL-6 increases correlate with mortality and initiation of systemic inflammatory response syndrome (SIRS) these results demonstrate that TP508 reduces systemic inflammatory response syndrome that was initiated by radiation or radiation combined with injury.

Example 8. Effect of TP508 on endothelial function as demonstrated by aortic explant endothelial cell sprouting assays.

To determine whether TP508 helped maintain endothelial function, an established angiogenesis assay was used. In these experiments mice were either non-irradiated or given exposures of 3 Gy, 8 Gy or 10 Gy. Approximately 2 hours post-exposure mice were injected IV with saline or saline plus TP508 (15 mg/kg). Mice were sacrificed 24 hours after exposure, aortas removed and aortic segments were placed on matrigel® and cultured in endothelial growth medium with growth supplement containing VEGF and FGF2 for 5 days. In the non-irradiated controls (0 Gy), TP508 more than doubled the amount of endothelial sprouting from the aortic segments during 5 day incubations as determined by measuring area occupied by sprouts or longest sprout projections. Aortic segment explants from 3 Gy exposed mice had some sprouting in the saline injected group, but again this sprouting was more than doubled in mice injected with TP508. In the 8 Gy and 10 Gy groups there was virtually no sprouting from aortic segments isolated from placebo mice, while visible sprouting continued to be observed at the edges of explants from T508-treated mice.

Example 9. Effect of TP508 on hematopoietic recovery and increases proliferation of bone marrow progenitor cells.

The bone marrow was isolated from non-exposed and mice exposed to 8 Gy with or without TP508 post-exposure injection. The samples were subjected to the complete blood count (CBC) analysis, which demonstrated earlier recovery of leukocyte, erythrocyte and thrombocyte numbers in mice treated with TP508. This result suggests that TP508 stimulates hematopoiesis or protects bone marrow cells (BMCs).

Histology of bone marrow 8 days after exposure to 8 Gy shows depletion of BMCs in marrow of 8 Gy exposed mice relative to 0 Gy mice. TP508 treatment of these mice increases

number and density of BMCS.

This finding was confirmed by EdU incorporation (DNA synthesis). At 12 days post-exposure, there is some new proliferation of BMCs representing a limited degree of recovery in 8 Gy exposed mice. In TP508-treated 8 Gy mice, 3-5 times more proliferating BMCs were observed. Thus, a single post-exposure injection of TP508 initiates a cascade of events that has restorative properties for BMCs.

CLAIMS

WHAT IS CLAIMED IS:

1. A method of reducing the risk of mortality in a subject exposed to a lethal dose of radiation, comprising administering to the subject an effective amount of a thrombin peptide derivative, wherein the thrombin peptide derivative comprises Asp-Ala-R, wherein R is a serine esterase conserved sequence.

2. The method of Claim 1, wherein the dose of radiation is at least 3.5 Gy.

3. The method of Claim 1 or 2, wherein the subject has additionally sustained traumatic injury, severe dermal injury and/or burn injury.

4. The method of Claim 3, wherein the traumatic injury is a fractured bone or an injury to an internal organ.

5. The method of Claim 3 or 4, wherein the burn injury, severe dermal injury or traumatic injury exposes the subject to systemic infection.

6. A method of treating a subject with traumatic injury, severe dermal injury and/or burn injury who is also exposed to radiation, comprising administering to the subject an effective amount of a thrombin peptide derivative, wherein the thrombin peptide derivative comprises Asp-Ala-R, wherein R is a serine esterase conserved sequence.

7. The method of Claim 6, wherein the radiation exposure is sub-lethal.

8. The method of Claim 7 wherein the radiation exposure is less than 3.5 Gy.

9. A method of reducing radiation related injury in a subject undergoing radiation therapy, comprising administering to the subject an effective amount of a thrombin peptide derivative, wherein the thrombin peptide derivative comprises Asp-Ala-R, wherein R is a serine esterase conserved sequence.

10. The method of Claim 9, wherein the radiation related injury is skin ulceration or late stage skin breakdown.

11. The method of Claim 9, wherein the radiation related injury is subcutaneous fibrosis.

12. The method of Claim 11, wherein the polypeptide is administered to normal tissue of the subject that is exposed or is to be exposed to the radiation.

13. The method of Claim 12, wherein the normal tissue that is exposed or is to be exposed to the radiation is skin.

14. The method of Claim 13, wherein the polypeptide is topically administered to the normal skin.

15. A method of reducing the risk of developing a radiation induced illness in a subject undergoing radiation therapy, comprising administering to the subject an effective amount of a thrombin peptide derivative, wherein the thrombin peptide derivative comprises Asp-Ala-R, wherein R is a serine esterase conserved sequence.

16. A method of promoting healing of a wound on a subject that was caused by

radiation exposure and or has been exposed to radiation, comprising administering to the wound an effective amount of a thrombin peptide derivative, wherein the thrombin peptide derivative comprises Asp-Ala-R, wherein R is a serine esterase conserved sequence and the thrombin peptide derivative has at least 20 amino acids.

17. The method of Claim 16, wherein the wound is a surgical wound.

18. The method of Claim 16, wherein the wound is a skin ulcer caused by the radiation.

19. The method of any one of Claims 1-18 or 48-67, wherein the thrombin peptide derivative is a polypeptide 12 to 23 amino acid residues in length.

20. The method of Claim 19, wherein the serine esterase conserved sequence comprises the polypeptide of Cys-X_1-Gly- Asp-Ser-Gly-Gly-Pro-X_2-Val (SEQ ID NO: 15), wherein X_1 is Glu or Gln and X_2 is Phe, Met, Leu, His or Val.

21. The method of Claim 20, wherein the serine esterase conserved sequence comprises Cys-Glu-Gly- Asp-Ser-Gly-Gly-Pro-Phe-Val (SEQ ID NO : 14).

22. The method of any one of Claims 19-21, wherein the thrombin peptide derivative comprises the polypeptide Arg-Gly-Asp-Ala (SEQ ID NO: 16).

23. The method of Claim 19, wherein the thrombin peptide derivative comprises the polypeptide Arg-Gly-Asp-Ala-Cys-X_1-Gly-Asp-Ser-Gly-Gly-Pro-X_2-Val (SEQ ID NO: 1), wherein X_1 is Glu or Gln and X2 is Phe, Met, Leu, His or Val.

24. The method of Claim 19, wherein the thrombin peptide derivative comprises the polypeptide Arg-Gly-Asp-Ala-Cys-Glu-Gly-Asp-Ser-Gly-Gly-Pro-Phe-Val (SEQ ID NO: 17).

25. The method of Claim 19, wherein the thrombin peptide derivative comprises the polypeptide Ala-Gly-Tyr-Lys-Pro-Asp-Glu-Gly-Lys-Arg-Gly-Asp-Ala-Cys-X_1-Gly-Asp-Ser-Gly-Gly-Pro-X_2-Val (SEQ ID NO:2), an N-terminal truncated fragment thereof having at least fourteen amino acid residues, or a C-terminal truncated fragment thereof having at least eighteen amino acid residues, wherein X_1 is Glu or Gln and X_2 is Phe, Met, Leu, His or Val.

26. The method of Claim 19, wherein the thrombin peptide derivative comprises the polypeptide Ala-Gly-Tyr-Lys-Pro-Asp-Glu-Gly-Lys-Arg-Gly-Asp-Ala-Cys-Glu-Gly-Asp-Ser-Gly-Gly-Pro-Phe-Val (SEQ ID NO:6), an N-terminal truncated fragment thereof having at least fourteen amino acid residues, or a C-terminal truncated fragment thereof having at least eighteen amino acid residues.

27. The method of Claim 19, wherein the thrombin peptide derivative comprises the polypeptide Ala-Gly-Tyr-Lys-Pro-Asp-Glu-Gly-Lys-Arg-Gly-Asp-Ala-Cys-Glu-Gly-Asp-Ser-Gly-Gly-Pro-Phe-Val (SEQ ID NO:6).

28. The method of any one of Claims 1-18 or 48-67, wherein the thrombin peptide derivative is H-Ala-Gly-Tyr-Lys-Pro-Asp-Glu-Gly-Lys-Arg-Gly-Asp-Ala-Cys-Glu-Gly-Asp-Ser-Gly-GIy-Pro-Phe-Val-NH_2 (SEQ ID NO:3).

29. The method of Claim 19, wherein the thrombin peptide derivative comprises the

polypeptide Arg-Gly-Asp-Ala-Xaa-X_1-Gly-Asp-Ser-Gly-Gly-Pro-X_2-Val (SEQ ID NO:4), wherein Xaa is alanine, glycine, serine or an S-protected cysteine; X_1 is Glu or Gln; and X_2 is Phe, Met, Leu, His or Val.

30. The method of Claim 19, wherein the thrombin peptide derivative comprises the polypeptide Arg-Gly-Asp-Ala-Xaa-Glu-Gly-Asp-Ser-Gly-Gly-Pro-Phe-Val (SEQ ID NO: 11), wherein Xaa is alanine, glycine, serine, or an S-protected cysteine.

31. The method of Claim 19, wherein the thrombin peptide derivative comprises the polypeptide Ala-Gly-Tyr-Lys-Pro-Asp-Glu-Gly-Lys-Arg-Gly-Asp-Ala-Xaa-X_1-Gly-Asp-Ser-Gly-Gly-Pro-X_2-Val (SEQ ID NO: 5) or a fragment thereof comprising amino acid residues 10-18 of SEQ ID NO:5, wherein Xaa is alanine, glycine, serine or an S-protected cysteine; X_1 is Glu or Gln; and X_2 is Phe, Met, Leu, His or Val.

32. The method of Claim 19, wherein the thrombin peptide derivative comprises the polypeptide Ala-Gly-Tyr-Lys-Pro-Asp-Glu-Gly-Lys-Arg-Gly-Asp-Ala-Xaa-Glu-Gly-Asp-Ser-Gly-Gly-Pro-Phe-Val (SEQ ID NO:20), or a fragment thereof comprising amino acid residues 10-18 of SEQ ID NO:20.

33. The method of any one of Claims 29-32, wherein Xaa is alanine or serine.

34. The method of Claim 19, wherein the thrombin peptide derivative is the polypeptide H-Ala-Gly-Tyr-Lys-Pro-Asp-Glu-Gly-Lys-Arg-Gly-Asp-Ala-Ala-Glu-GIy-Asp-Ser-Gly-Gly-Pro-Phe-Val-NH_2 (SEQ ID NO:22) or H-Ala-Gly-Tyr-Lys-Pro-Asp-Glu-Gly-Lys-Arg-Gly-Asp-Ala-Ser-Glu-Gly-Asp-Ser-Gly-Gly-Pro-

Phe-Val-NH_2 (SEQ ID NO:30).

35. The method of any one of Claims 19-27 or 29-34, wherein the thrombin derivative comprises a C- terminal amide and optionally comprises an acylated N-terminus, wherein said C-terminal amide is represented by -$C(O)NR_aR_b$, wherein R_a and Rb are independently hydrogen, an aliphatic group comprising up to 10 carbon atoms, or R_a and R_b, taken together with the nitrogen to which they are bonded, form a C_3-C_{10} non-aromatic heterocyclic group, and said N-terminal acyl group is represented by $R_0C(O)$-, where Ro is hydrogen, an aliphatic group comprising up to 10 carbon atoms or a phenyl group optionally substituted with one or more groups selected from C_1-C_6 alkyl, C_1-C_6 alkoxy, C_1-C_6 haloalkyl, C_1-C_6 haloalkoxy, nitro and cyano.

36. The method of Claim 45, wherein the thrombin peptide derivative comprises an N-terminus which is unsubstituted, and a C-terminus which is unsubstituted or a C-terminal amide represented by -$C(O)NH_2$.

37. The method of any one of Claims 1-18 or 48-67, wherein the thrombin peptide derivative is a polypeptide dimer, wherein each polypeptide in the dimer is from 12 to 23 amino acid residues in length and wherein each polypeptide in the dimer independently comprises Asp-Ala-Cys-X_1-Gly-Asp-Ser-Gly-Gly-Pro-X_2-Val (SEQ ID NO: 10), wherein X_1 is Glu or Gln and X_2 is Phe, Met, Leu, His or Val, said polypeptides optionally comprising a C-terminal amide; and said polypeptides optionally comprising an acylated *N*-terminus.

38. The method of Claim 37, wherein each polypeptide in the dimer is the same.

39. The method of Claim 38, wherein each polypeptide in the dimer is covalently linked through a disulfide bond.

40. The method of Claim 39, wherein each polypeptide in the dimer comprises Arg-Gly-Asp-Ala-Cys-X_1-Gly-Asp-Ser-Gly-Gly-Pro-X_2-Val (SEQ ID NO: 1), wherein X_1 is Glu or Gln and X_2 is Phe, Met, Leu, His or Val.

41. The method of Claim 39, wherein each polypeptide in the dimer comprises Arg-Gly-Asp-Ala-Cys-Glu-Gly-Asp-Ser-Gly-Gly-Pro-Phe-Val (SEQ ID NO: 17).

42. The method of Claim 39, wherein each polypeptide in the dimer comprises Ala-Gly-Tyr-Lys-Pro-Asp-Glu-Gly-Lys-Arg-Gly-Asp-Ala-Cys-X_1-Gly-Asp-Ser-Gly-Gly-Pro-X_2-Val (SEQ ID NO:2), wherein X, is Glu or Gln and X_2 is Phe, Met, Leu, His or Val or a fragment thereof comprising amino acid residues 10-18 of SEQ ID NO:2.

43. The method of Claim 39, wherein each polypeptide in the dimer comprises Ala-Gly-Tyr-Lys-Pro-Asp-Glu-Gly-Lys-Arg-Gly-Asp-Ala-Cys-Glu-Gly-Asp-Ser-Gly-Gly-Pro-Phe-Val (SEQ ID NO:6), or a fragment thereof comprising amino acid residues 10-18 of SEQ ID NO:6.

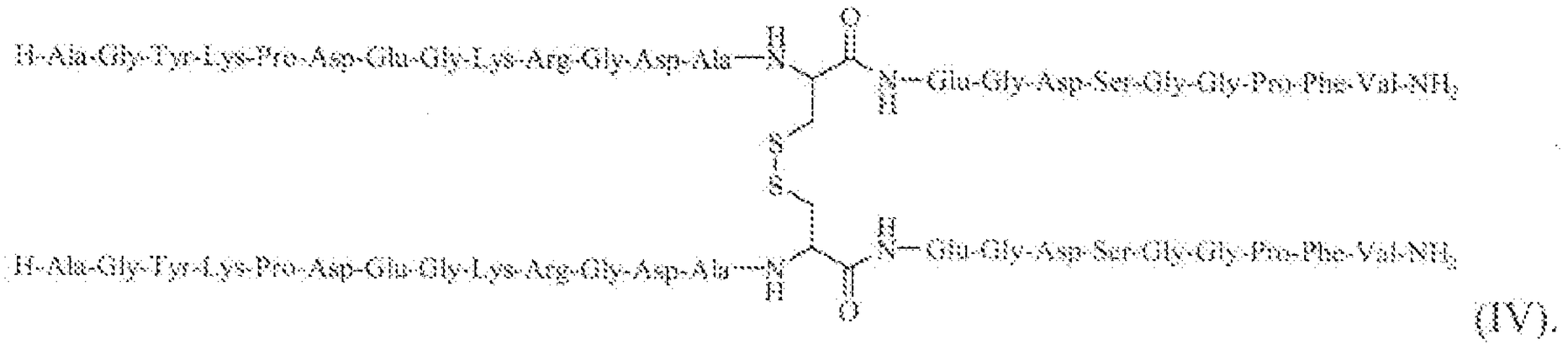
44. The method of Claim 39, wherein each polypeptide in the dimer comprises Ala-Gly-Tyr-Lys-Pro-Asp-Glu-Gly-Lys-Arg-Gly-Asp-Ala-Cys-Glu-Gly-Asp-Ser-Gly-Gly-Pro-Phe-Val (SEQ ID NO:6).

45. The method of any one of Claims 38-44, wherein each polypeptide in the dimer comprises a C- terminal amide and optionally comprises an acylated N-terminus,

wherein said C-terminal amide is represented by -$C(O)NR_2R_b$, wherein R_3 and R_b, are independently hydrogen, an alkyl group comprising up to 10 carbon atoms, or R_3 and R_b, taken together with the nitrogen to which they are bonded, form a C_3-C_{10} non-aromatic heterocyclic group, and said N-terminal acyl group is represented by $R_0C(O)$-, where R_0 is hydrogen, an alkyl group comprising up to 10 carbon atoms or a phenyl group optionally substituted with one or more groups selected from C_1-C_6 alkyl, C_1-C_6 alkoxy, C_1-C_6 haloalkyl, C_1-C_6 haloalkoxy, nitro and cyano.

46. The method of Claim 45, wherein each polypeptide in the dimer comprise an N-terminus which is unsubstituted; and a C-terminus which is unsubstituted or a C-terminal amide represented by -$C(O)NH_2$.

47. The method of Claim 39, wherein the thrombin peptide derivative is represented by the following structural formula:



(IV).

48. A method of reducing the risk of developing a bacterial, fungal or viral infection in the blood of a subject exposed to a bacterial, fungal or viral infection, comprising administering to the subject an effective amount of a thrombin peptide derivative, wherein the thrombin peptide derivative comprises Asp-Ala-R,

wherein R is a serine esterase conserved sequence.

49. The method of Claim 48, wherein the subject has sustained exposure to radiation.

50. The method of Claim 49, wherein the subject has sustained breakdown of the intestinal wall from the radiation.

51. The method of Claim 49 or 50, wherein the radiation exposure is sub-lethal.

52. The method of any one of Claim 49 or 50, wherein the subject is exposed to a lethal dose of radiation.

53. The method of any one of Claims 48-52, wherein the subject has sustained traumatic injury, severe dermal injury and/or burn injury.

54. The method of any one of Claims 48-52, wherein the subject has been exposed to the infection during or within 30 days after an invasive medical or dental procedure.

55. The method of Claim 54, wherein the invasive medical procedure is surgery.

56. The method of Claim 48, wherein the subject has pneumonia or other pulmonary diseases

57. The method of Claim 48, wherein the subject is immunocompromised.

58. The method of Claim 57, wherein the subject has AIDS.

59. The method of Claim 57, wherein the subject has cancer.

60. The method of any one of Claims 48-59, wherein the subject is older than 60 years old.

61. The method of any one of Claims 48-59, wherein the subject is an infant.

62. The method of any one of Claims 48-61, wherein an invasive medical device is introduced into the subject.

63. The method of claim 62, wherein the said invasive medical device includes an intravenous or an arterial line, a breathing tube, a urinary catheter, a surgical drain or an artificial joint.

64. The method of any one of Claims 48-62, wherein the thrombin peptide derivative is introduced in combination with another therapeutic agent.

65. The method of Claim 64, wherein the agent is an antibiotic.

66. The method of Claim 64, wherein the thrombin peptide derivative is administered intravenously.

67. A method of reducing the risk of developing systemic inflammatory response syndrome (SIRS) in a subject exposed to a bacterial, fungal or viral infection, comprising administering to the subject an effective amount of a thrombin peptide derivative, wherein the thrombin peptide derivative comprises Asp-Ala-R, wherein R is a serine esterase conserved sequence.

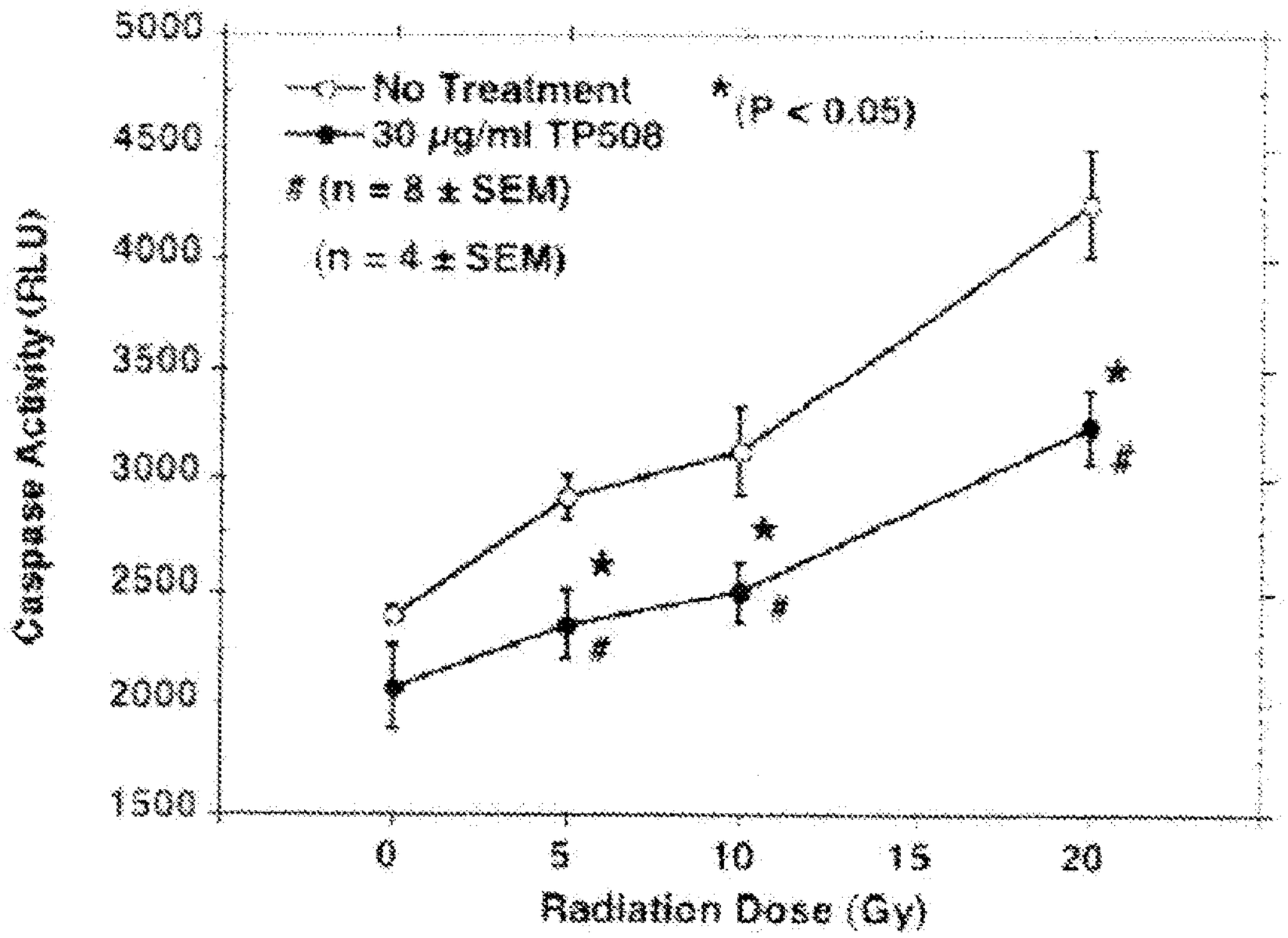

No Treatment
30 µg/ml TP508
* (n = 8 ± SEM)
(n = 4 ± SEM)
* (P < 0.05)
Caspase Activity (RLU)
5000
4500
4000
3500
3000
2500
2000
1500
0
5
10
15
20
Radiation Dose (Gy)

Figure 1.

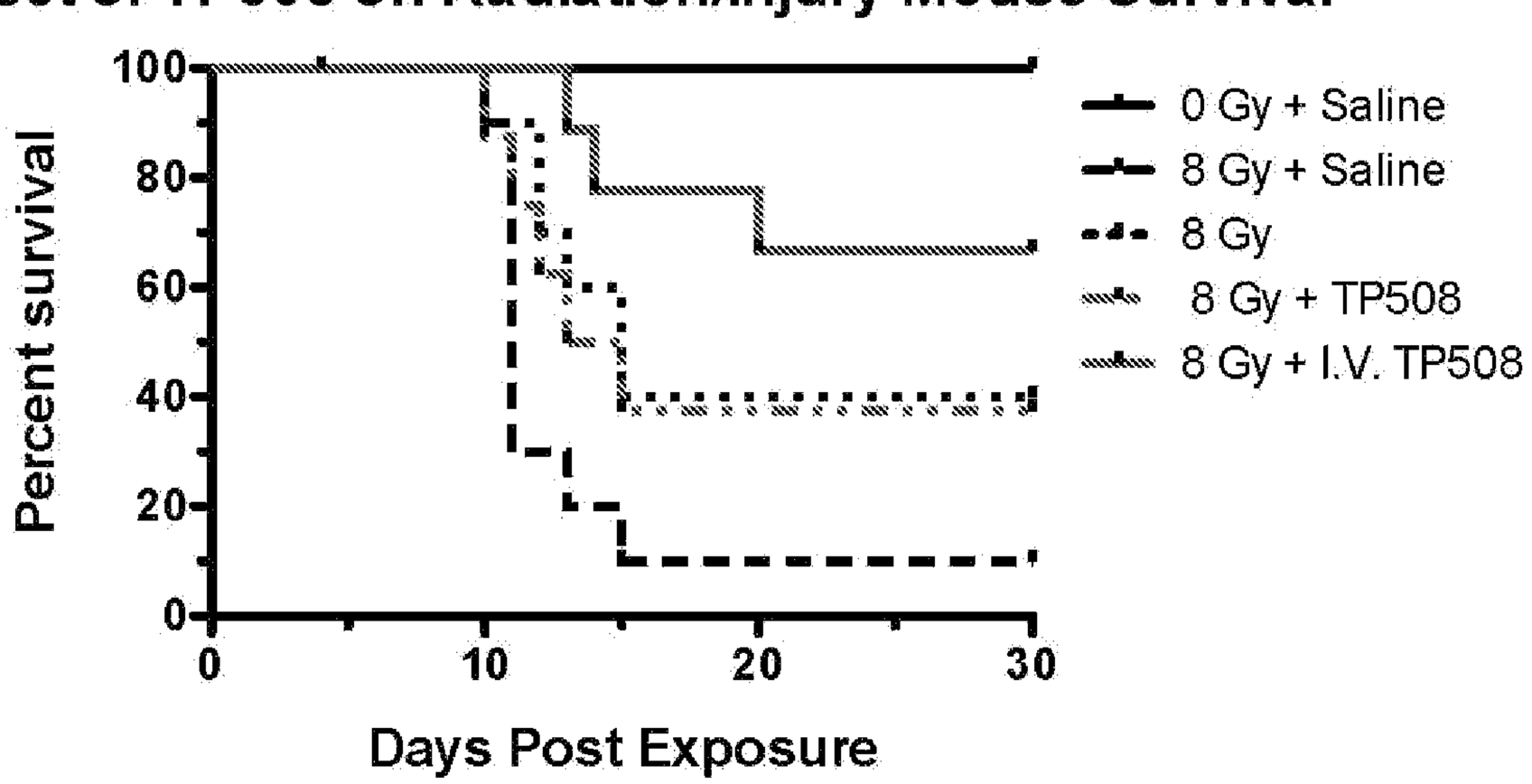
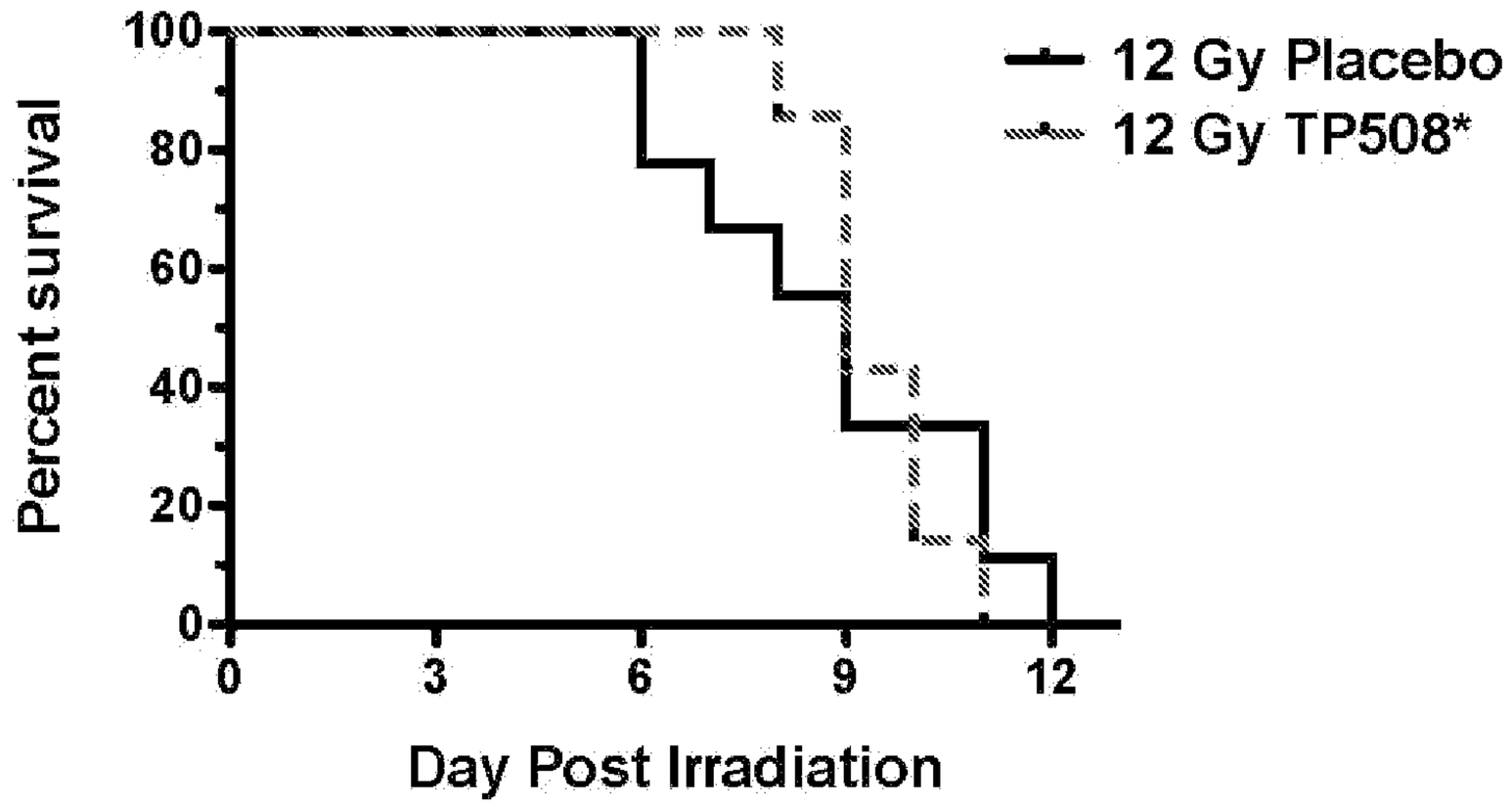

Effect of TP508 on Radiation/Injury Mouse Survival
Percent survival
100
80
60
40
20
0
0
10
20
30
Days Post Exposure
0 Gy + Saline
8 Gy + Saline
8 Gy
8 Gy + TP508
8 Gy + I.V. TP508

Figure 2.

Effect of Post-Exposure Injection of TP508 on Mouse Survival - High Lethal Dose (12 Gy)



* N = 10 mice per group
TP508 shows "Strong Trend" toward extending survival. p = 0.052

Figure 3.

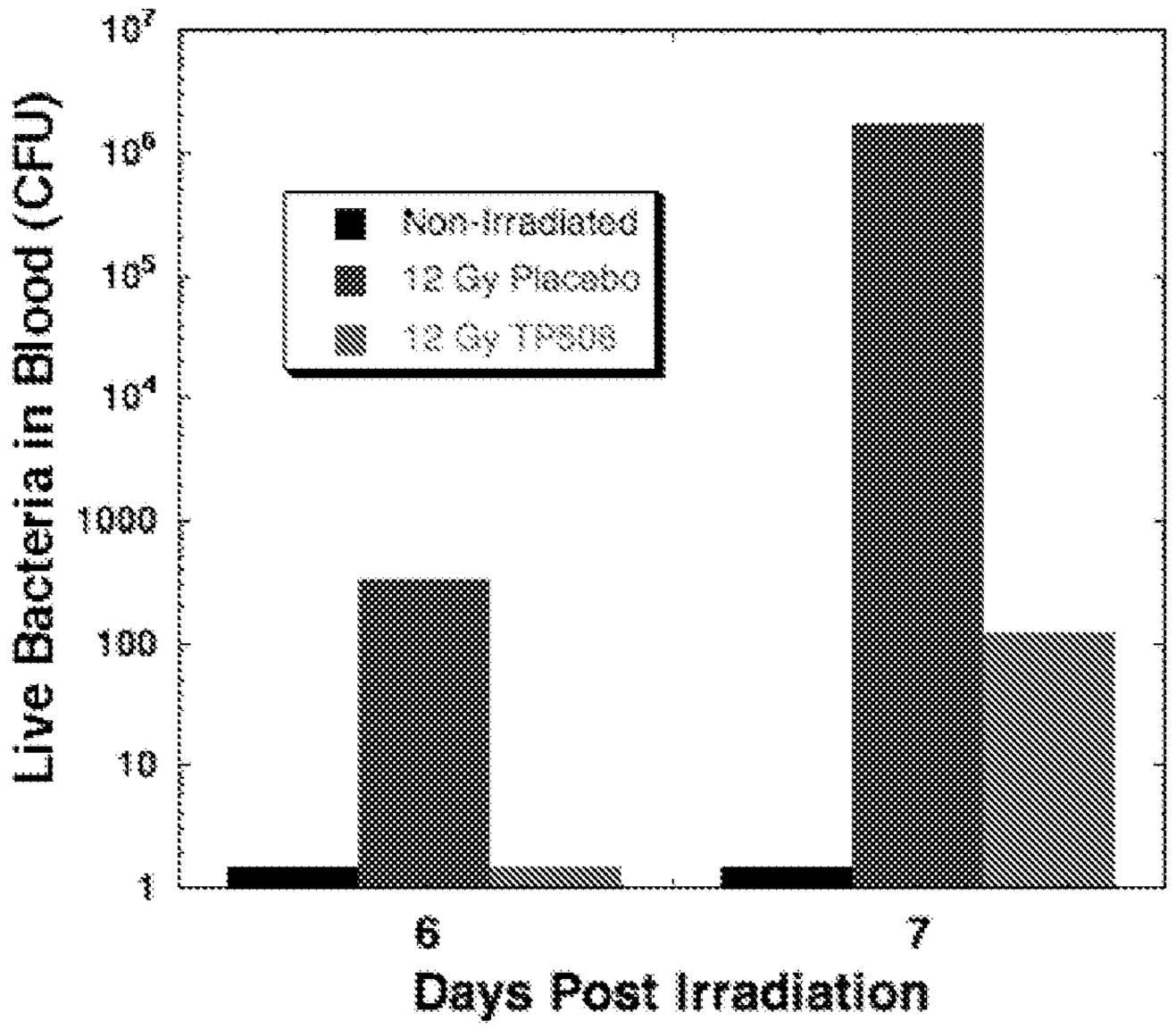

Live Bacteria in Blood (CFU)
10^7
10^6
10^5
10^4
1000
100
10
1
Non-Irradiated
12 Gy Placebo
12 Gy TP508
6
7
Days Post Irradiation

Figure 4.

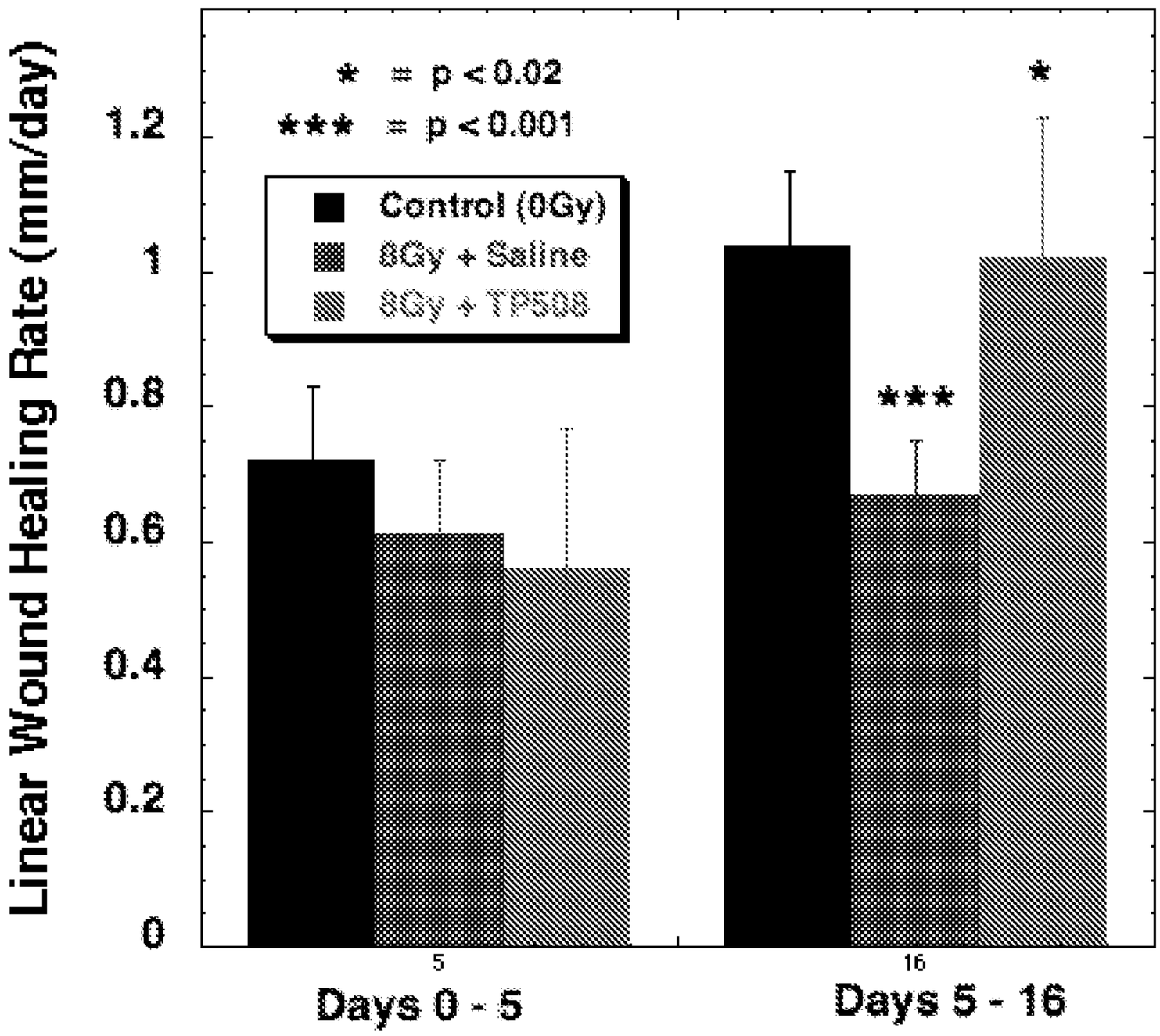

Effect of Radiation and TP508 on LWH Rate
* = p < 0.02
*** = p < 0.001
Control (0Gy)
8Gy + Saline
8Gy + TP508
Linear Wound Healing Rate (mm/day)
1.2
1
0.8
0.6
0.4
0.2
0
*
***
*
5
16
Days 0 - 5
Days 5 - 16

Figure 5.

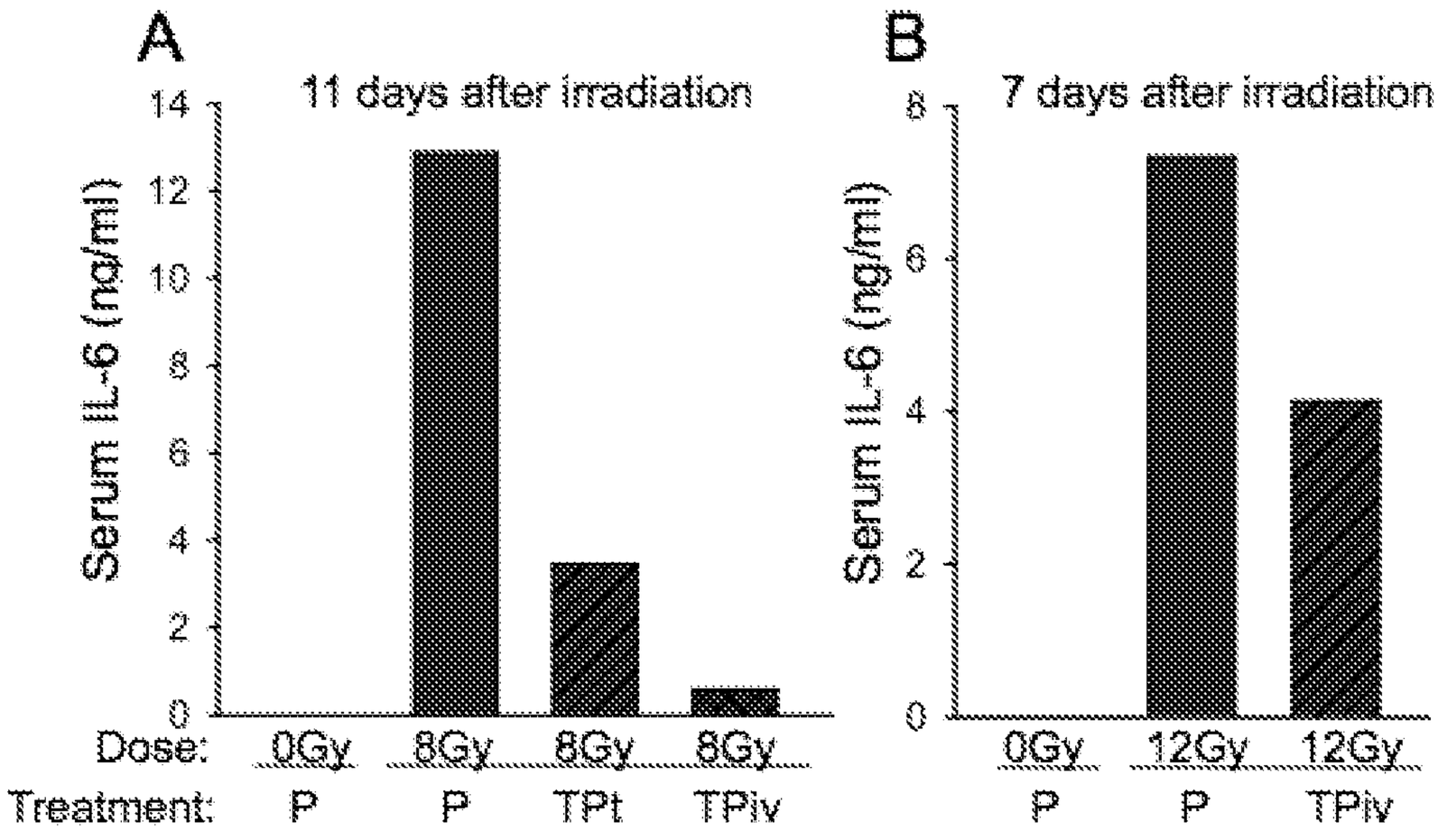

A
11 days after irradiation
Serum IL-6 (ng/ml)
0
2
4
6
8
10
12
14
Dose:
0Gy
8Gy
8Gy
8Gy
Treatment:
P
P
TPt
TPiv
B
7 days after irradiation
Serum IL-6 (ng/ml)
0
2
4
6
8
Dose:
0Gy
12Gy
12Gy
Treatment:
P
P
TPiv

Figure 6.

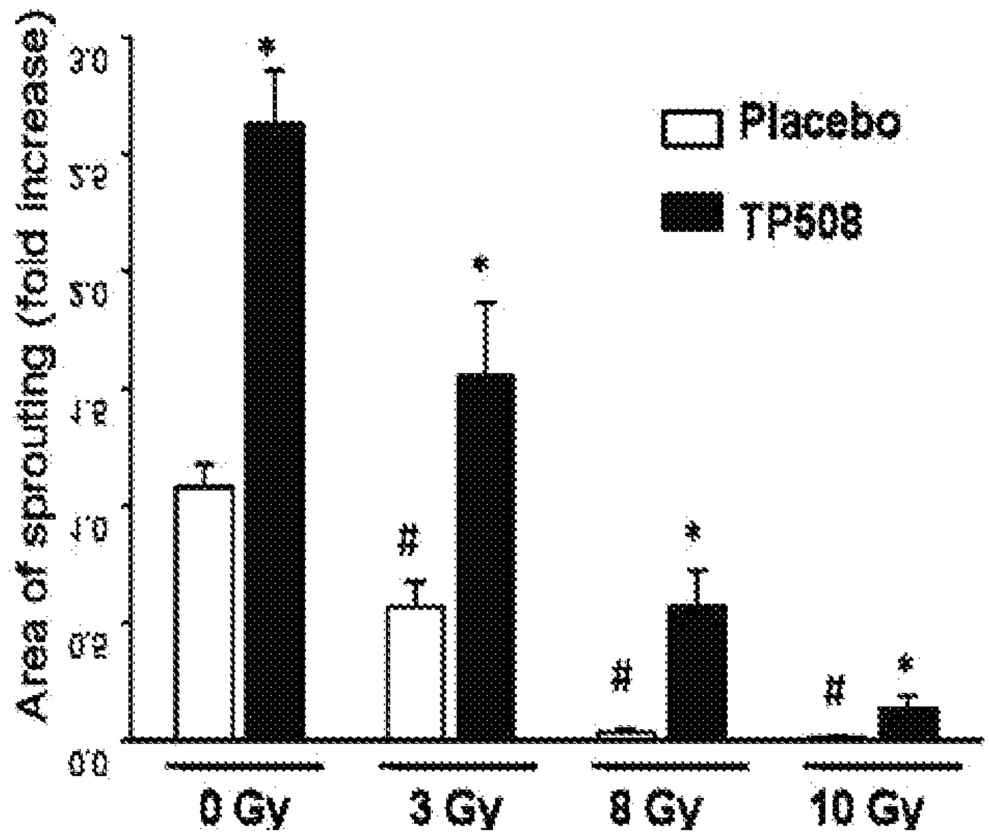

Area of sprouting (fold increase)
Placebo
TP508
0 Gy
3 Gy
8 Gy
10 Gy
*
#

Figure 7.

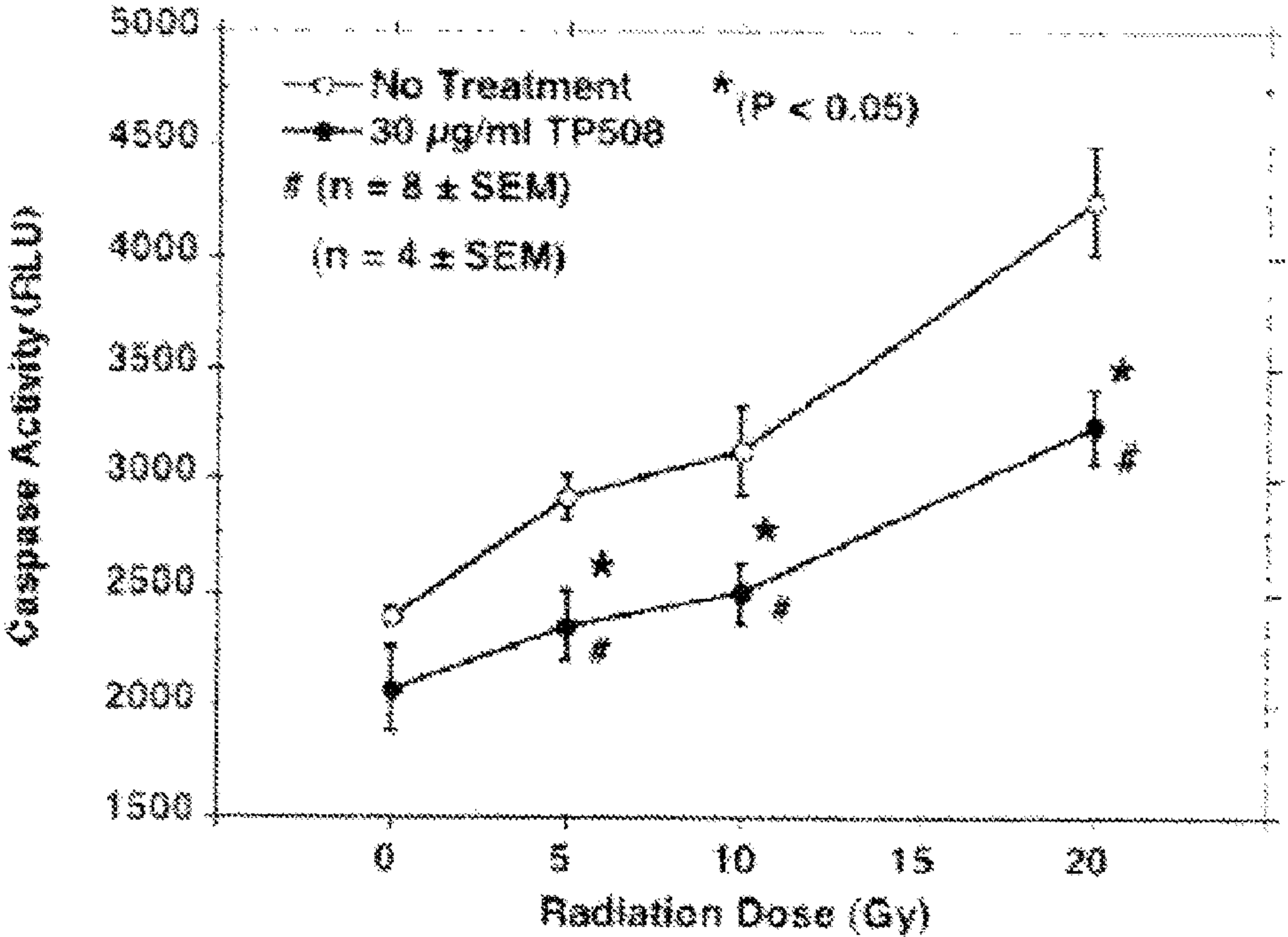

No Treatment
30 µg/ml TP508
* (n = 8 ± SEM)
(n = 4 ± SEM)
*(P < 0.05)
Caspase 8/8 Activity (RLU)
5000
4500
4000
3500
3000
2500
2000
1500
0
5
10
15
20
Radiation Dose (Gy)

Figure 1.