COMPOSITIONS AND METHODS FOR ENHANCING SYSTEMIC DELIVERABILITY, TOLERABILITY, AND EFFICACY OF CATIONIC MACROCYCLIC PEPTIDES

[0001] This application claims the benefit of United States Provisional Application No. 62/743,243, filed on October 9, 2018. These and all other referenced extrinsic materials are incorporated herein by reference in their entirety. Where a definition or use of a term in a reference that is incorporated by reference is inconsistent or contrary to the definition of that term provided herein, the definition of that term provided herein is deemed to be controlling.

Field of the Invention

[0002] The field of the invention is enhancement of systemic deliverability or pharmacologic effect of cationic peptide drugs, in particular enhancement of macrocyclic θ-defensins and/or analogs thereof.

Background

[0003] The following description includes information that may be useful in understanding the present invention. It is not an admission that any of the information provided herein is prior art or relevant to the presently claimed invention, or that any publication specifically or implicitly referenced is prior art.

[0004] Inflammation is a complex protective response to pathogens, tissue damage, and exposure to irritants, involving alteration in blood vessels, mobilization of immune cells, and release of a variety of chemical and peptide mediators. While inflammation serves to remove the initial cause of cell injury and eliminate necrotic cells from damaged tissue, the inflammatory response can itself be damaging. For example, chronic inflammation resulting from autoimmune disease can contribute to damage of the affected tissue. Similarly, inflammation resulting from acute processes, such as viral or bacterial infection, can result in tissue damage and septic shock, and this may be exacerbated by chronic inflammation when the initial insult results in prolonged and unresolved inflammation. Chronic inflammation often leads to tissue scarring, fibrosis, and loss of function. In addition, the pain and swelling that accompany inflammation can be debilitating, particularly when it is the result of chronic conditions.

[0005] Unfortunately, current methods for treating inflammation suffer from a number of drawbacks. For example, traditional pharmaceutical approaches (*e.g.* treatment with steroids or non-steroidal inflammatory drugs) provide only short term relief, and often do so at the cost of significant side effects that limit the use of such drugs. More recently, "biologics" (for example, humanized monoclonal antibodies to proinflammatory cytokines) have been used to treat certain chronic conditions characterized by inflammation, however such approaches necessarily target only a single inflammation mechanism, and can result in immune suppression or even an immunocompromised state in a treated individual. In addition, antibody-based biologics can require the intravenous administration of relatively large volumes of fluid, which is generally administered by infusion while under the care of trained medical personnel.

[0006] Mammalian defensins are cationic, tri-disulfide-containing peptides comprising three structurally distinct and different subfamilies. The α and β defensins are linear peptides that range in length from 29 to about 60 amino acids, with free amino and carboxyl terminal amino acids. The α- and β-defensins have similar three dimensional topologies but differ in their disulfide linkages (Selsted and Ouellette, Nat Immunol 6:551-557 (2005)). In contrast, naturally occurring θ-defensins are peptide backbone-cyclized 18-amino acid peptides that are both structurally and functionally distinct from α and β defensins. Defensins contribute to host defense as antimicrobial agents (Ericksen *et al*, Antimicrob Agents Chemother 49:269-275 (2005)) and by regulating inflammatory (Khine *et al*, Blood 107:2936-2942 (2006)) and adaptive immune responses (Chertov *et al*, J Biol Chem 271:2935-2940 (1996)). All publications herein are incorporated by reference to the same extent as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference. Where a definition or use of a term in an incorporated reference is inconsistent or contrary to the definition of that term provided herein, the definition of that term provided herein applies and the definition of that term in the reference does not apply.

[0007] θ-defensins are expressed in Old World monkeys (e.g., macaques and baboons), and are the only known cyclic proteins in animals (Lehrer *et al*, J Biol Chem 287:27014-27019 (2012)). The basic θ-defensin backbone structure is produced by head-to-tail splicing of two nonapeptide precursors. In rhesus macaques alternate binary splicing of nonapeptides encoded by three precursor genes provides six θ-defensin isoforms, rhesus theta-defensins RTD-1 to RTD-6 (SEQ

ID NOs. 1 to 6) (Tang *et al*, Science 286:498-502 (1999); Leonova *et al*, J Leukoc Biol 70:461-464 (2001)). In baboons, alternate nonapeptide splicing produces ten θ-defensin isoforms, baboon theta defensins BTD-1 to BTD-10 (SEQ ID Nos. 7 to 16) (Garcia *et al*, Infect Immun 76:5883-5891 (2001)). θ-defensins are expressed at high levels in granules of neutrophils and in monocytes of these species. These θ-defensins play a major role in the antimicrobial activities of rhesus neutrophil granule extracts. The RTD-1 isoform is the most abundant θ-defensin in macaques, constituting approximately 55% of the total θ-defensin content of rhesus neutrophils (Tongaonkar *et al*, J Leukoc Biol 89:283-290 (2011)).

[0008] Humans and other hominids lack θ-defensins due to the presence of a stop codon mutation in the prepro-coding sequence of θ-defensin genes in these species (Nguyen *et al*, Peptides 24:1647-1654 (2003)). It has been suggested that the expression of θ-defensins in Old World monkeys is related to differences in immune and inflammatory responses of these nonhuman primates from those of humans (Lehrer *et al*, J Biol Chem 287:27014-27019 (2012)).

[0009] While α-, β-, and θ-defensins were initially identified on the basis of broad spectrum antimicrobial properties, subsequent studies have disclosed different and distinct immune regulatory roles (Yang *et al*, Annu Rev Immunol 22:181-215 (2004)). For example, some α- and β-defensins are chemotactic for T cells, neutrophils, dendritic cells, and monocytes (Chertov *et al*, J Biol Chem 271:2935-2940 (1996); Yang *et al*, Science 286:525-528 (1999); Grigat *et al,* J Immunol 179:3958-3965 (2007); Soruri *et al*, Eur J Immunol 37:2474-2486 (2007)), and induce secretion of proinflammatory cytokines from activated dendritic cells, peripheral blood mononuclear cells and epithelial cells (Khine *et al*, 107:2936-2942 (2006); Boniotto *et al*, Antimicrob Agents Chemother 50:1433-1441 (2006); Ito *et al*, Tohoku J Exp Med 227:39-48 (2012); Yin *et al*, Immunol 11:37. (2010); Niyonsaba *et al*, J Immunol 175:1776-1784 (2005); Li *et al*, Invest Ophthalmol Vis Sci 50:644-653 (2009); Syeda *et al*, J Cell Physiol 214:820-827 (2008)).

[0010] In contrast to such pro-inflammatory activities, it has recently been reported that θ-defensins have anti-inflammatory properties both *in vitro* and *in vivo*. For example, RTD-1 was found to be a potent inhibitor of cytokine secretion by human peripheral blood leukocytes stimulated with diverse Toll-like receptor (TLR) agonists (Schaal *et al*, PLoS One 7, e51337

(2012)). Naturally occurring θ-defensin isoforms (RTDs 1 to 6) possess variable potency in reducing TNF in lipopolysaccharide- or *E. coli*-stimulated leukocytes. (Schaal *et al*, PLoS One 7, e51337 (2012)). RTD-1 has also been found to reduce inflammatory cytokines, including TNF-α, IL-1β, and several chemokines in mouse models of SARS coronavirus infection (Wohlford-Lenane *et al*, J Virol 83:11385-11390 (2009), in *E. coli* peritonitis, and in polymicrobial sepsis (Schaal *et al*, PLoS One 7, e51337 (2012)).

[0011] Recently, Selsted and Tran (United States Patent Application Publication No. 2013/0157964) have shown that θ-defensins and θ-defensin analogs that retain θ-defensin core structures are effective in treating chronic inflammatory conditions, such as rheumatoid arthritis. It is unclear, however, what formulations of these small cyclic peptides are optimal for achieving therapeutic efficacy at drug exposure low enough to limit potential adverse events, including those associated with injection site reactions following subcutaneous administration.

[0012] International Patent Application Publication No. WO 02/064166, to Jeong *et al*., describes a method for improving pharmacodynamic effect of some peptide drugs by forming an emulsion in the presence of a surfactant and an organic solvent in an aqueous solution, then removing the organic solvent prior to administration. Various alcohols and polyols, including propylene glycol, are cited as suitable organic solvents that are removed prior to administration. It is not clear, however, if this approach would be effective with a small, basic, cyclic peptide such as a θ-defensin.

[0013] United States Patent Application Publication No. 2006/084605 (to Engelud *et al.*) and United States Patent Application Publication No. 2006/0287221 (to Knudsen *et al.*) describe the use of various polyols in peptide pharmaceutical formulations for parenteral administration as isotonicity agents. Similarly, United States Patent Application Publication No. 2007/0010424 (to Pedersen *et al.*) describes the use of propylene glycol in place of various sugars as an isotonicity agent, in order to prevent the formation of deposits that can interfere with the function of equipment and needles utilized in the administration of various specified peptide drugs. The reference, however, does not document any effect beyond the reduction in solid residue provided by such formulations, and there is no evidence or suggestion that the use of such compounds had any effect on the bioavailability or pharmacodynamic effects of the peptide drugs so prepared.

[0014] International Patent Application Publication No WO 2016/059593 (to Sonavaria *et al.*) describes injectable formulations for peptide drugs that contain high (up to 99%) concentrations of organic solvents (including polyols), which are described as improving the stability of the peptides. Injection of high concentrations of such organic solvents, however, is associated with significant negative effects, including pain and swelling.

[0015] Thus, there is still a need for methods and compositions that provide a pharmacodynamic effect for administered defensins adequate to achieve sufficient therapeutic effect.

Summary of The Invention

[0016] The inventive subject matter provides compositions and methods that provide injectable formulations for θ-defensins and θ-defensin analogs, and which provide improved bioavailability relative to conventional isotonic saline solutions. Such formulations also have low viscosity, permitting scalable sterilization by filtration.

[0017] One embodiment of the inventive concept is a method of treating an individual with a chronic inflammatory condition (such as rheumatoid arthritis, inflammatory bowel disease, inflammation associated with cancer, diabetes, and/or a chronic disease characterized by dysregulated or unresolved chronic inflammation) by providing a θ-defensin or θ-defensin analog as an aqueous solution comprising the θ -defensin or θ-defensin analog and from 0.5% to 1.5% v/v propylene glycol and having a pH of from 5.0 to 7.0, and administering the aqueous solution by subcutaneous injection to an individual in need of treatment. In some embodiments the aqueous solution includes an acetate salt. The aqueous solution provides an increase in pharmacologic potency or therapeutic effect of the θ-defensin or θ-defensin analog relative to a solution of the θ-defensin or θ-defensin analog in normal saline. The pharmacodynamic effect of the θ-defensin or θ-defensin analog can be increased by at least 10-fold to 40-fold relative to a similar concentration of the θ-defensin or θ-defensin analog provided in a normal saline solution. The θ-defensin or θ-defensin analog can be provided at concentrations up to 50 $mgmL^{-1}$. A θ-defensin analog utilized in the method can be a cyclic icosipeptide, a cyclic enneadecapeptide, a cyclic octadecapeptide, a cyclic heptadecapeptide, a cyclic hexadecapeptide, a cyclic pentadecapeptide, a cyclic tetradecapeptide, a cyclic tridecapeptide, a cyclic dodecapeptide, a

cyclic hendecapeptide, or a cyclic decapeptide. In a preferred embodiment the aqueous solution includes 1% v/v propylene glycol and 20mM acetate, and has a pH of 6.0.

[0018] Another embodiment of the inventive concept is a method of sterilizing an aqueous θ-defensin preparation by providing a θ-defensin or θ-defensin analog in an aqueous buffer that includes the θ-defensin or θ-analog at a concentration of at least 1 $mgmL^{-1}$ to 50 $mgmL^{-1}$ (or higher) and propylene glycol at from 0.5% to 1.5% v/v, and passing the aqueous buffer through a filter having a pore size of 0.2μm or less. The aqueous buffer can include an acetate salt, and the resulting θ-defensin preparation can have a pH of 5.0 to 7.0.

[0019] Another embodiment of the inventive concept is a pharmaceutical composition for treatment of a chronic inflammatory condition (such as rheumatoid arthritis, inflammatory bowel disease, inflammation associated with cancer, diabetes, and/or a chronic disease characterized by dysregulated or unresolved chronic inflammation) that includes a θ-defensin or θ-defensin analog at up to 50 $mgmL^{-1}$ and propylene glycol at from 0.5% to 1.5% v/v, and can include an acetate salt. The pharmaceutical composition can have a pH of from 5.0 to 7.0 and provides an increase in pharmacodynamic effect of the θ-defensin or θ-defensin analog relative to a corresponding pharmaceutical composition prepared without propylene glycol on parenteral administration. The pharmacologic potency of the θ-defensin or θ-defensin analog is increased by at least 10-fold to 40-fold relative to an identical concentration of the θ-defensin or θ-defensin analog provided in a normal saline solution. Such a pharmaceutical composition can be formulated for subcutaneous injection, intramuscular injection, and/or intravenous injection. A suitable θ-defensin analog can be a cyclic icosipeptide, a cyclic enneadecapeptide, a cyclic octadecapeptide, a cyclic heptadecapeptide, a cyclic hexadecapeptide, a cyclic pentadecapeptide, a cyclic tetradecapeptide, a cyclic tridecapeptide, a cyclic dodecapeptide, a cyclic hendecapeptide, or a cyclic decapeptide. In a preferred embodiment the pharmaceutical composition includes 1% v/v propylene glycol and 20mM acetate, and has a pH of 6.0.

[0020] Another embodiment of the inventive concept is the use of a θ-defensin or θ-defensin analog at a concentration of up to 50 $mgmL^{-1}$ and in a solution containing propylene glycol at from 0.5% to 1.5% v/v for treatment of a chronic inflammatory condition (such as rheumatoid arthritis, inflammatory bowel disease, inflammation associated with cancer, diabetes, and/or a

chronic disease characterized by dysregulated or unresolved chronic inflammation). The solution can include an acetate salt. The solution is formulated for parenteral administration, can have a pH of from 5.0 to 7.0, and provides increased pharmacodynamic effect of the θ-defensin or θ-defensin analog relative to a corresponding amount of θ-defensin or θ-defensin analog prepared without propylene glycol. Pharmacologic potency of the θ-defensin or θ-defensin analog can be increased by at least 10-fold to 40-fold on parenteral administration relative to a similar concentration of the θ-defensin or θ-defensin analog provided in a normal saline solution. Suitable routes for parenteral administration include subcutaneous injection, intramuscular injection, and/or intravenous injection. Suitable θ-defensin analogs include a cyclic icosipeptide, a cyclic enneadecapeptide, a cyclic octadecapeptide, a cyclic heptadecapeptide, a cyclic hexadecapeptide, a cyclic pentadecapeptide, a cyclic tetradecapeptide, a cyclic tridecapeptide, a cyclic dodecapeptide, a cyclic hendecapeptide, and/or a cyclic decapeptide. In a preferred embodiment the composition includes 1% v/v propylene glycol and 20mM acetate, and has a pH of 6.0.

[0021] Another embodiment of the inventive concept is use of a θ-defensin or θ-defensin analog at up to 50 $mgmL^{-1}$ and propylene glycol at from 0.5% to 1.5% v/v for preparation of a composition useful for treatment of a chronic inflammatory condition (such as rheumatoid arthritis, inflammatory bowel disease, inflammation associated with cancer, diabetes, and/or a chronic disease characterized by dysregulated or unresolved chronic inflammation). The solution can include an acetate salt. The composition is formulated for parenteral administration, can have a pH of from 5.0 to 7.0, and provides increased pharmacodynamic effect of the θ-defensin or θ-defensin analog relative to a corresponding amount of θ-defensin or θ-defensin analog prepared without propylene glycol. Pharmacologic potency of the θ-defensin or θ-defensin analog can be increased by at least 10-fold to 40-fold on parenteral administration relative to a similar concentration of the θ-defensin or θ-defensin analog provided in a normal saline solution. Suitable routes for parenteral administration include subcutaneous injection, intramuscular injection, and/or intravenous injection. Suitable θ-defensin analogs include a cyclic icosipeptide, a cyclic enneadecapeptide, a cyclic octadecapeptide, a cyclic heptadecapeptide, a cyclic hexadecapeptide, a cyclic pentadecapeptide, a cyclic tetradecapeptide, a cyclic tridecapeptide, a cyclic dodecapeptide, a cyclic hendecapeptide, and/or a cyclic

decapeptide. In a preferred embodiment the composition includes 1% v/v propylene glycol and 20mM acetate, and has a pH of 6.0.

[0022] Various objects, features, aspects and advantages of the inventive subject matter will become more apparent from the following detailed description of preferred embodiments, along with the accompanying drawing figures in which like numerals represent like components.

Brief Description of The Drawings

[0023] FIG. 1: FIG. 1 shows typical data demonstrating the effects of subcutaneous injection of RTD-1 in saline on an animal model of rheumatoid arthritis.

[0024] FIG. 2: FIG. 2 shows typical effects of subcutaneous injection of RTD-1 in saline containing 1% (v/v) propylene glycol on an animal model of rheumatoid arthritis.

[0025] FIG. 3: FIG. 3 shows typical effects of subcutaneous injection of RTD-1 in saline containing 1% (v/v) propylene glycol or 20 mM Na acetate containing 1% (v/v) propylene glycol on an animal model of rheumatoid arthritis.

[0026] FIGs. 4A and 4B: FIGs. 4A and 4B show graphs of plasma θ-defensin concentration (in ng/mL) vs time (in hours) for male and female rats, respectively, on day 1 of a study in which a θ-defensin formulation of the inventive concept is injected subcutaneously.

[0027] FIGs. 5A and 5B: FIGs. 5A and 5B show graphs of plasma θ-defensin concentration (in ng/mL) vs time (in hours) for male and female rats, respectively, on day 13 of a study in which a θ-defensin formulation of the inventive concept is injected subcutaneously.

[0028] FIGs. 6A and 6B: FIGs. 6A and 6B show graphs of plasma θ-defensin concentration (in ng/mL) vs time (in hours) for male and female rats, respectively, on day 41 of a study in which a θ-defensin formulation of the inventive concept is injected subcutaneously.

[0029] FIGs. 7A and 7B: FIG. 7A provides a graph depicting results of a C_{max} vs. θ-defensin dose linearity study for male and female rats at day 1of a study in which a θ-defensin formulation of the inventive concept is injected subcutaneously. FIG. 7B provides a graph depicting results

of a C_{max} vs. θ-defensin dose linearity study for male and female rats at day 41 of a study in which a θ-defensin formulation of the inventive concept is injected subcutaneously.

[0030] FIGs. 8A and 8B: FIG. 8A provides a graph depicting results of an $AUC_{0\text{-}TLast}$ vs. θ-defensin dose linearity study for male and female rats at day 1of a study in which a θ-defensin formulation of the inventive concept is injected subcutaneously. FIG. 8B provides a graph depicting results of an $AUC_{0\text{-}TLast}$ vs. θ-defensin dose linearity study for male and female rats at day 41 of a study in which a θ-defensin formulation of the inventive concept is injected subcutaneously.

[0031] FIG. 9: FIG. 9 shows typical results for measurement of θ-defensin concentration (ng/mL) in plasma over time for different treatment groups in a human clinical trial in which a θ-defensin formulation of the inventive concept was injected subcutaneously.

[0032] FIGs. 10A and 10B: FIG. 10A shows a typical dependence of C_{max} (ng/mL) on dose (μg/kg) of a θ-defensin formulation of the inventive concept that was injected subcutaneously in a human clinical trial. FIG. 10B shows a typical dependence of $AUC_{0\text{-}TLast}$ on dose (μg/kg) of a θ-defensin formulation of the inventive concept that was injected subcutaneously in a human clinical trial.

[0033] FIGs. 11A and 11B: FIG. 11A shows a typical dependence of C_{max} (ng/mL) on total θ-defensin dose (mg) of a θ-defensin formulation of the inventive concept that was injected subcutaneously in a human clinical trial. FIG. 11B shows a typical dependence of $AUC_{0\text{-}TLast}$ on total θ-defensin dose (mg) of a θ-defensin formulation of the inventive concept that was injected subcutaneously in a human clinical trial.

Detailed Description

[0034] The following discussion provides many example embodiments of the inventive subject matter. Although each embodiment represents a single combination of inventive elements, the inventive subject matter is considered to include all possible combinations of the disclosed elements. Thus, if one embodiment comprises elements A, B, and C, and a second embodiment comprises elements B and D, then the inventive subject matter is also considered to include other remaining combinations of A, B, C, or D, even if not explicitly disclosed.

[0035] The inventive subject matter provides apparatus, systems and methods in which propylene glycol at from 0.5% to 1.5% in a slightly acidic (*e.g.* pH of 5 to 7) aqueous solution has, surprisingly, been found to increase the preclinical efficacy of a parenterally administered θ-defensin and/or θ-defensin analog by at least 20-fold relative to conventional neutral isotonic saline solutions. This permits convenient subcutaneous administration of θ-defensin in relatively small volumes (*e.g.* about 1 mL) in amounts that are effective in treating chronic inflammatory conditions such as rheumatoid arthritis, inflammatory bowel disease, or diabetes, or other conditions resulting from dysregulated inflammatory responses in humans. Addition of propylene glycol at these concentrations was also unexpectedly found to dramatically reduce the viscosity of concentrated (*e.g.* 10 $mgmL^{-1}$ or higher) solutions of θ-defensin, permitting simple, convenient, and scalable sterilization by filtration. Further, the formulation of θ-defensin markedly reduced injection site reactions relative to those observed when isotonic saline was used as the formulation vehicle.

[0036] Suitable θ-defensins include native θ-defensins found in mammals expressing these peptides, and can also include one or more θ-defensins derived from untranslated genes present in some primate species (*e.g. Homo sapiens*). In some embodiments of the inventive concept one or more θ-defensin can correspond to those found in *Macaca mulatta*, for example RTD-1 (SEQ ID NO. 1), RTD-2 (SEQ ID NO. 2), RTD-3 (SEQ ID NO. 3), RTD-4 (SEQ ID NO. 4), RTD-5 (SEQ ID NO. 5), and/or RTD-6 (SEQ ID NO. 6). In other embodiments of the inventive concept one or more θ-defensin can correspond to those found in *Papio anubis*, for example BTD-1 (SEQ ID NO. 7), BTD-2 (SEQ ID NO. 8), BTD-3 (SEQ ID NO. 9), BTD-4 (SEQ ID NO. 10), BTD-5 (SEQ ID NO. 11), and/or BTD-6 (SEQ ID NO. 12), BTD-7 (SEQ ID NO. 13), BTD-8 (SEQ ID NO. 14), BTD-9 (SEQ ID NO. 15), and/or BTD-10 (SEQ ID NO. 16).

[0037] Within this application embodiments describing θ-defensins and uses thereof are inclusive of θ-defensin analogs. The term θ-defensin analog refers to a cyclic peptide having about 40%, 50%, 60%, 70%, 80%, 90% or greater sequence identity with a native θ-defensin peptide sequence. A θ-defensin analog can incorporate one, two, three, or more core features of a native θ-defensin. Exemplary core features include cyclic structure, the presence of one, two, three, or more disulfide bonds within the peptide (e.g. between pairs of cysteines of the analog),

having a positive charge when in solution under physiological conditions, and the presence of beta pleated sheet secondary structure. Such θ-defensin analogs can include 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more than 20 amino acids, and in some embodiments can incorporate non-naturally occurring amino acids. An analog of a θ-defensin can include one or more L-amino acid(s), one or more D-amino acid(s), and/or a mixture of L- and D- amino acids. In some embodiments non-peptide bonds can be utilized between adjacent amino acid residues of a θ-defensin analog. θ-defensin analogs can represent one or more deletion or substitution of amino acids of a native θ-defensin sequence. Such substitutions can be conservative (e.g. where the substituted amino acid(s) retain(s) charge, hydrophobicity, hydrophilicity, and/or steric properties of the native amino acid). In some embodiments θ-defensin analogs can include grafting or conjugation of non-peptide moieties, for example polyethylene glycol and/or other hydrophilic polymers, cell-receptor targeting moieties, and/or moieties that aid in processing/purification. Examples of suitable theta defensin analogs that are based on RTD-1 (SEQ ID NO. 1) are provided as SEQ ID NO. 17 (RTD-1-27), SEQ ID NO. 18 (RTD-1-28), and SEQ ID NO. 19 (RTD-1-29).

[0038] Compositions and methods for enhancing the pharmacologic effect of a θ-defensin and/or a θ-defensin analog utilized in the treatment of chronic inflammatory conditions (*e.g.* rheumatoid arthritis and/or diabetes) are provided herein. Such compositions incorporate from about 0.5% to about 1.5% propylene glycol in a mildly acidic aqueous solution (*e.g.* about pH 5 to 7), and are suitable for subcutaneous injection of θ-defensin at a concentration of up to 50 $mgmL^{-1}$. Such formulations have, surprisingly, been found to dramatically (*e.g.* greater than at least 10-fold) increase in pharmacologic effect or potency of the administered θ-defensin relative to θ-defensin provided at the same or similar concentrations in conventional saline solutions and/or solutions that do not include propylene glycol. This is shown in FIGs. 1, 2, and 3 below. It has also been found that such formulations have greatly reduced viscosity relative to θ-defensin provided in conventional normal saline solutions (*e.g.* phosphate buffered saline, pH 7 to 7.5) at similar concentrations, which permits sterilization by filtration. Further, compositions claimed markedly reduce injection site reactions compared to isotonic saline vehicle.

[0039] FIGs. 1, 2, and 3 show the results of treatment of an animal model of rheumatoid arthritis by subcutaneous injection of the θ-defensin RTD-1, where the θ-defensin is provided in either a conventional normal saline vehicle or in normal saline containing a low concentration (1% v/v) of propylene glycol. Rats with established pristane induced arthritis were treated with daily subcutaneous injections of RTD-1 formulated in normal saline at the indicated doses (FIG. 1) or in a saline solution containing 1% propylene glycol (FIG. 2). The lowest effective RTD-1 dose in the normal saline diluent was 1 mg/kg, with maximal apparent effect seen at 3 mg/kg (see FIG. 1). The inclusion of 1% propylene glycol in the vehicle markedly lowered the effective RTD-1 dose (to 0.08 mg/kg, see FIG. 2) in that 0.08 mg/kg (the lowest dose tested) produced a maximal anti-arthritic effect, equivalent to 3 mg/kg doses of RTD-1 in saline (i.e. the maximum observed effect). Equivalent improvement in pharmacologic effect was obtained when RTD-1 was formulated in a buffered (20 mM sodium acetate) aqueous diluent containing 1% propylene glycol (FIG. 3). The Applicant believes that similar effects can be achieved at lower doses of the θ-defensin.

[0040] Surprisingly, it has also been found that such formulations have greatly reduced viscosity relative to θ-defensin provided in conventional normal saline solutions (*e.g.* isotonic phosphate buffered saline, pH 7 to 7.5) at similar concentrations, which permits sterilization by filtration.

[0041] Inventors have previously noted that θ-defensins and θ-defensin analogs (for example, cyclic octadecapeptides and/or cyclic tetradecapeptides) have significant effects in reducing inflammation in animal models of chronic inflammatory disease, such as pristane-treated rats. θ-defensins are readily soluble in aqueous solutions. In initial studies the θ-defensin was prepared in conventional normal saline solutions and was readily administered by subcutaneous injection with no apparent systemic ill effects in mice, though at high θ-defensin concentrations fat necrosis in the subcutaneous tissue was observed. Similarly, in rat and canine animal models, low concentrations of θ-defensins were well tolerated systemically and locally. However, at higher concentrations, injections of θ-defensin in normal saline resulted in localized inflammation and swelling at the injection site in a dose-dependent manner. Swelling and inflammation were found to persist for weeks. Relatively high concentrations of θ-defensin (*e.g.*

about 10 to 50 $mgmL^{-1}$), however, are likely to be necessary to provide the necessary θ-defensin dosage while maintaining volumes appropriate for subcutaneous injection in human therapy.

[0042] Microscopic studies of injection sites where θ-defensin had been administered in conventional saline solution showed localized inflammation and necrotic changes at injection sites of canine and porcine test subjects. Without wishing to be bound by theory, Inventors believe that θ-defensin may be interacting with components of the extracellular matrix found within the skin of some mammals at subcutaneous injection depths, causing it to precipitate and/or form precipitating complexes within this tissue layer. Studies of the interaction of RTD-1 with whole blood, blood plasma collected following treatment with anticoagulant, and serum indicate that the θ-defensin interacts with fibrinogen to form insoluble complexes. Inventors believe that θ-defensin may, therefore, be interacting with fibrinogen and/or fibrinogen-like proteins associated with the extracellular matrix.

[0043] Inventors found that use of hypotonic saline, addition of poloxamers, and the use of other conventional excipients failed to reduce or eliminate this θ-defensin induced injection site inflammation and tissue injury. Surprisingly, low concentrations of propylene glycol were effective in providing solubility for high concentrations (*e.g.* 10 $mgmL^{-1}$ or greater) of θ-defensin and also in reducing or preventing swelling and/or inflammation on subcutaneous injection of high concentrations of θ-defensin into susceptible species. Propylene glycol is freely miscible with water, available as a sterile liquid of pharmaceutical grade, and is generally recognized as safe. The effective range of propylene glycol concentrations for reduction and/or elimination of adverse reactions on injection of high concentrations of θ-defensin was found to be relatively narrow. In some embodiments the effective concentration of propylene glycol in water was found to be from about 0.4% to about 1.6% (v/v). In other embodiments the effective concentration of propylene glycol in water was found to be about 0.5% to about 1.5% (v/v). In a preferred embodiment the effective concentration of propylene glycol in water is about 1% (v/v).

[0044] Further studies showed that, surprisingly, results were further improved by using a propylene glycol/water solvent with mildly acidic pH (*e.g.* from about pH 5 to 7). In a preferred embodiment the pH of the propylene glycol/water solvent is about 6.0, and has a final osmolality of about 180 to 230 mOsmoles. The pH of the propylene glycol/water solvent can be maintained

using a buffer species at low molarity (*e.g.* less than about 50 mM). Suitable buffer concentrations can, for example, range from 1 mM to 50 mM, 5 mM to 35 mM, 10 mM to 30 mM, or about 20 mM. Suitable buffer species can be salts of organic acids, such as acetate, citrate, malate, tartrate, HEPES, MES, etc. In other embodiments the buffer species can be a zwitterionic species having a suitable pKa. In a preferred embodiment the buffer species is an acetate salt (e.g. sodium acetate) at a concentration of about 20 mM, and provides a pH of about 6.0. Surprisingly, Inventors have found that the use of saline (*e.g.* NaCl) in even small (*e.g.* less than 50 mM) amounts results in adverse reactions to subcutaneous injection of θ-defensin. Preferred embodiments of the aqueous solvent system used for subcutaneous administration can exclude NaCl and similar salts.

[0045] Surprisingly, Inventors have found that θ-defensins are freely soluble in such acidic propylene glycol/water solvent systems, and that only transient dermal reactions are found following subcutaneous injections of RTD-1 in rats and dogs at concentrations of up to 12.5 $mgmL^{-1}$, and with only minor adverse effects at 50 $mgmL^{-1}$. This supports the estimated 10 $mgmL^{-1}$ concentration of θ-defensin expected as necessary for providing dosing adequate for human treatment.

[0046] As noted above, Inventors unexpectedly found that use of a propylene glycol/water solvent system, in addition to eliminating the inflammation associated with saline-based formulations, dramatically increases the pharmacologic potency of a θ-defensin administered subcutaneously relative to conventional saline formulations. The increase in preclinical efficacy in established pristane-induced arthritis in mildly acidic propylene glycol/water relative to similar amounts provided in conventional saline solutions can be at least 5-fold, 10-fold, 15-fold, 20-fold, 30-fold, 40-fold, 50-fold, or more. For example, subcutaneous injection of RTD-1 provided at 0.08 mg/kg in 1% propylene glycol + 20 mM acetate, pH 6, was found to have an effect equivalent to the administration of RTD-1 at 3 mg/kg in isotonic saline in the relief of symptoms of rheumatoid arthritis. This suggests an increase in pharmacologic potency of at least 37-fold, however since the relief of symptoms provided was at the upper limit of the benefit afforded by θ-defensins administered in isotonic saline it is probable that the actual increase in pharmacodynamic effect is greater than 37-fold (e.g. greater than 40-fold, 50-fold, 70-fold, 100-

fold, or higher). This advantageously reduces the amount of θ-defensin necessary to provide adequate treatment.

[0047] Sterilization of conventional solutions utilized for injection is commonly performed by filtration, for example using a filter having a pore size of 0.2 μm or less. This process is, however, problematic for protein drug solutions as such solutions are typically too viscous for efficient sterile filtration at the high protein concentrations that are desirable to provide small injection volumes. As used herein, such a viscous solution refers to a solution or dispersion in which the internal resistance to flow is so high that filtration is difficult or impossible (e.g. requiring pressures exceeding the burst pressure of the filtration membrane). The viscosity of such a viscous solution can be as high as 105 centipoise (cp) or higher. In one embodiment the viscosity of such a viscous solution is at least about 90 to about 95 cp. In another embodiment the viscosity of such a viscous solution is at least about 40 cp. In still another embodiment the viscosity of such a viscous solution is at least above the viscosity of water (i.e. above about 1.0 cP).

[0048] During the course of investigation Inventors found that, while θ-defensins are highly soluble, aqueous solutions can become highly viscous as protein concentration increases. In practice conventional saline solutions of 2% w/v θ-defensin or higher were found to be too viscous to permit sterilization by filtration at a reasonable and/or manufacturable scale. Surprisingly, similar solutions prepared in propylene glycol/water solvents have low viscosity at high (*e.g.* 2% w/v, 5% w/v, 10% w/v, or greater) θ-defensin concentrations. This permits simple and scalable sterilization of such preparations using conventional filtration through media having a pore size of about 0.2 μm or less.

[0049] Embodiments of the inventive concepts include methods of treating conditions associated with chronic inflammation by subcutaneous injection of a drug composition that includes one or more θ-defensin(s) in an acidic aqueous solution containing propylene glycol at from 0.5% to 1.5%. Suitable conditions include rheumatoid arthritis, inflammatory bowel disease, diabetes, and other conditions resulting from dysregulation inflammatory responses. The drug composition can include a single species of θ-defensin or two or more species of θ-defensins. The drug composition can be administered subcutaneously in a volume ranging from about 0.1

mL to about 2.5 mL, about 0.25 mL to about 2 mL, 0.5 mL to about 1.5 mL, or about 1 mL. The concentration of θ-defensin or total concentration of species of θ-defensins in the drug composition can range from about 1 $mgmL^{-1}$ to about 50 $mgmL^{-1}$, and is preferably about 12.5 $mgmL^{-1}$ or less. Concentration of θ-defensin and/or injection volume can be adjusted to provide a dose of from about 0.001 mg/kg to about 3 mg/kg, about 0.01 to about 1 mg/kg, or about 0.08 mg/kg.

[0050] In some embodiments the drug composition can include, in addition to one or more θ-defensin(s) and/or θ-defensin analog(s) and propylene glycol, additional therapeutic compounds. For example, the drug composition can include one or more steroids having an anti-inflammatory effect, non-steroidal anti-inflammatory drug(s), antibody(ies) or antibody fragment(s) directed to a pro-inflammatory cytokine, and/or one or analgesic compound(s).

[0051] Such preparations of θ-defensin can be administered using any suitable schedule. Subcutaneous injections can be provided at a frequency of once a week, twice a week, three times a week, alternating days, daily, every 12 hours, every 8 hours, or every 6 hours, as necessary to establish or maintain a desired therapeutic effect. Length of treatment can range from about 1 week, about 2 weeks, about 4 weeks, about 8 weeks, about 12 weeks, about 16 weeks, about 20 weeks, about 6 months, about 12 months, about 18 months, about 24 months, or greater than 24 months, as necessary to establish or maintain a desired therapeutic effect. In some embodiments dosing with θ-defensin can be higher and/or more frequent during initial stages of treatment in order to establish remission or partial remission of symptoms, then reduced in terms of either or both of θ-defensin dose and/or frequency of administration in order to maintain the remission of symptoms.

Examples

Pharmacokinetic studies in rats

[0052] The θ-defensin RTD-1 was formulated in a mildly acidic buffer containing propylene glycol as described above, and administered subcutaneously to male and female Sprague-Dawley rats followed by determination of plasma levels of the θ-defensin. Rats received doses ranging from 1 to 4 mg/kg. Dose volume was held constant at 0.32 mL/kg; accordingly, concentration of

the θ-defensin ranged from 3.125 to 12.5 mg/mL. Doses were administered three times per week for six weeks. Exemplary results are shown in Table 1 (which provides results for male rats) and Table 2 (which provides results for female rats). Table 1 and the following tables use the following acronyms:

$AUC_{0\text{-}TLast}$	Area under the plasma concentration versus time curve from time 0 (pre-dose) to the last measurable concentration time point
C_{Last}	Last plasma concentration measured above the limit of quantitation
C_{max}	Maximum plasma concentration
MRT_{Last}	Mean residence time up to the last time point when plasma concentrations of analyte were measured
T_{Last}	Time at which the last plasma concentration above the limit of quantification was measured
T_{max}	Time of maximum concentration.

TABLE 1

Group and Dose	C_{max} (ng/mL)[1]	T_{max} (hr)[1]	$AUC_{0-TLast}$ (ng*hr/mL)	T_{Last} (hr)	C_{Last} (ng/mL)	MRT_{Last} (hr)
2) RTD-1 Low Dose (1 mg/kg/dose)	**Day 1**					
	14.951	12	159	12	14.951	6.4
	Day 13					
	74.644	4.0	951	24	13.369	10.0
	Day 41					
	164.846	12	2158	24	17.766	9.8
3) RTD-1 Mid Dose (2 mg/kg/dose)	**Day 1**					
	26.635	0.5	319	24	13.747	11.8
	Day 13					
	29.429	0.5	512	24	23.851	12.6
	Day 41					
	208.296	12	3462	24	80.526	10.8
4) RTD-1 High Dose (4 mg/kg/dose)	**Day 1**					
	28.403	0.5	451	24	22.182	12.7
	Day 13					
	54.720	0.5	776	24	35.863	12.6
	Day 41					
	435.68	24	8103	24	435.680	13.1

[1] The apparent C_{max} and T_{max} values were determined for blood sampling times between pre-dose and 24 hours post dosing. There was no blood sampling between 12 and 24 hours. On Day 41, one highest mean plasma concentration in the High Dose group was found at the 24-hour blood sampling time point, indicating that it was possible that T_{max} was achieved between 12 and 24 hours.

TABLE 2

Group and Dose	C_{max} (ng/mL)[1]	T_{max} (hr)[1]	$AUC_{0-TLast}$ (ng*hr/mL)	T_{Last} (hr)	C_{Last} (ng/mL)	MRT_{Last} (hr)
2) RTD-1 Low Dose (1 mg/kg/dose)	**Day 1**					
	18.901	0.5	357	24	14.579	12.2
	Day 13					
	97.675	24	1215	24	97.675	15.4
	Day 41					
	176.959	0.5	2133	24	144.416	13.8
3) RTD-1 Mid Dose (2 mg/kg/dose)	**Day 1**					
	20.997	12	400	24	13.849	12.0
	Day 13					
	37.786	24	636	24	37.786	14.4
	Day 41					
	296.145	12	4675	24	127.434	11.5
4) RTD-1 High Dose (4 mg/kg/dose)	**Day 1**					
	37.129	12	743	24	36.851	13.6
	Day 13					
	177.471	12	2364	24	51.198	11.5
	Day 41					
	305.575	12	6138	24	263.958	12.6

[1] The apparent C_{max} and T_{max} values were determined for blood sampling times between pre-dose and 24 hours post dosing. There was no blood sampling between 12 and 24 hours. On Day 41, one highest mean plasma concentration in the High Dose group was found at the 24-hour blood sampling time point, indicating that it was possible that T_{max} was achieved between 12 and 24 hours.

[0053] Tables 3, 4, and 5 show typical results from kinetic studies of plasma concentration of the θ-defensin (in ng/mL) in male rats receiving 1mg/kg, 2 mg/kg, and 4 mg/kg, respectively. Tables 6, 7, and 8 show typical results from similar studies performed in female rats. FIGs. 4A and 4B show graphs of plasma θ-defensin concentration (in ng/mL) vs time (in hours) for male and female rats, respectively, on day 1 of such a study. FIGs. 5A and 5B show graphs of plasma θ-defensin concentration (in ng/mL) vs time (in hours) for male and female rats, respectively, on day 13 of such a study. FIGs. 6A and 6B show graphs of plasma θ-defensin concentration (in ng/mL) vs time (in hours) for male and female rats, respectively, on day 41 of such a study.

TABLE 3

Group		Sampling Time							
		Pre-dose	Time Post Dosing Relative to Pre-dose						
			30 min	1 hr	2 hr	4 hr	8 hr	12 hr	24 hr
2-TK RTD-1 Low Dose (1 mg/kg/dose)	**Day 1**								
	Mean	BLOQ	13.302	13.614	BLOQ	12.464	BLOQ	14.951	BLOQ
	SD	N/A	2.630	3.329	N/A	N/A	N/A	N/A	N/A
	%CV	N/A	20	24	N/A	N/A	N/A	N/A	N/A
	N*	0	3	2	0	1	0	1	0
	Day 13								
	Mean	18.660	21.697	52.084	13.177	74.644	14.283	64.415	13.369
	SD	N/A	6.701	69.303	5.121	88.833	2.196	87.142	2.164
	%CV	N/A	31	133	39	119	15	135	16
	N	1	3	3	3	2	3	3	3
	Day 41								
	Mean	74.847	26.314	148.135	21.294	152.933	24.763	164.846	17.766
	SD	97.607	8.487	181.751	10.080	179.737	7.553	208.874	5.992
	%CV	130	32	123	47	118	31	127	34
	N	3	3	3	3	3	3	3	3

BLOQ – Below the limit of quantitation, < 10 ng/mL; N represents the number of rats reporting with RTD-1 concentrations above the limit of quantitation.
Values are presented as the mean ± SD of N = 3 and the average ± the range of N = 2.
N/A - not applicable.

Group		TABLE 4						
		Sampling Time						
		Pre-dose	Time Post Dosing Relative to Pre-dose					
			30 min	1 hr	2 hr	4 hr	8 hr	12 hr	24 hr
3-TK RTD-1 Mid Dose (2 mg/kg/dose)		Day 1							
	Mean	BLOQ	26.635	22.732	13.967	11.224	14.136	11.934	13.747
	SD	N/A	1.214	15.855	0.404	1.575	0.892	2.022	2.301
	%CV	N/A	5	70	3	14	6	17	17
	N	0	3	3	2	3	3	2	3
		Day 13							
	Mean	BLOQ	29.429	19.115	19.560	16.807	**23.740**	20.622	**23.851**
	SD	N/A	12.833	0.819	3.122	1.755	5.215	1.842	2.561
	%CV	N/A	44	4	16	10	22	9	11
	N	0	3	3	3	3	3	3	3
		Day 41							
	Mean	135.977	92.475	190.915	75.016	200.315	93.949	208.296	80.526
	SD	114.179	59.333	143.297	45.629	157.574	44.884	179.603	44.818
	%CV	84	64	75	61	79	48	86	56
	N	3	3	3	3	3	3	3	3

BLOQ – Below the limit of quantitation, < 10 ng/mL; N represents the number of rats reporting with RTD-1 concentrations above the limit of quantitation.
Values are presented as the mean ± SD of N = 3 and the average ± the range of N = 2.
For N = 3, **bold** values are significantly different from Day 1; pair-wise comparison, $p < 0.05$ Student's t-test.
N/A - not applicable.

Group		TABLE 5						
		Sampling Time						
		Pre-dose	Time Post Dosing Relative to Pre-dose					
			30 min	1 hr	2 hr	4 hr	8 hr	12 hr	24 hr
4-TK RTD-1 High Dose (4 mg/kg/dose)		Day 1							
	Mean	BLOQ	28.403	17.401	14.295	16.338	22.693	16.141	22.182
	SD	N/A	10.138	3.699	4.245	3.656	5.658	3.924	4.199
	%CV	N/A	36	21	30	22	25	24	19
	N	0	3	3	3	2	3	3	3
		Day 13							
	Mean	10.875	**54.720**	25.465	**28.070**	22.843	38.772	30.731	35.863
	SD	0.745	12.698	6.083	1.916	2.595	10.251	10.583	12.180
	%CV	7	23	24	7	11	26	34	34
	N	2	3	3	3	3	3	3	3
		Day 41							
	Mean	231.831	386.809	289.317	334.089	276.001	356.169	283.309	435.680
	SD	140.748	360.928	190.507	367.103	161.971	363.267	154.004	533.685
	%CV	61	93	66	110	59	102	54	122
	N	3	3	3	3	3	3	3	3

BLOQ – Below the limit of quantitation, < 10 ng/mL; N represents the number of rats reporting with RTD-1 concentrations above the limit of quantitation.
Values are presented as the mean ± SD of N = 3 and the average ± the range of N = 2.
For N = 3, **bold** values are significantly different from Day 1; pair-wise comparison, $p < 0.05$ Student's t-test.
N/A - not applicable.

TABLE 6

Group		Sampling Time							
		Pre-dose	Time Post Dosing Relative to Pre-dose						
			30 min	1 hr	2 hr	4 hr	8 hr	12 hr	24 hr
2-TK RTD-1 Low Dose (1 mg/kg/dose)	**Day 1**								
	Mean	BLOQ	18.901	15.549	12.780	12.731	14.212	16.814	14.579
	SD	N/A	7.198	2.879	N/A	1.960	4.152	2.611	6.260
	%CV	N/A	38	19	N/A	15	29	16	43
	N	0	3	3	1	3	2	3	3
	Day 13								
	Mean	BLOQ	43.692	11.859	65.055	12.849	78.536	18.469	97.675
	SD	N/A	43.430	0.935	76.310	1.092	114.621	1.051	136.032
	%CV	N/A	99	8	117	9	146	6	139
	N	0	3	3	2	2	3	3	3
	Day 41								
	Mean	17.879	176.959	20.237	154.096	22.465	156.429	29.272	144.416
	SD	N/A	274.447	13.515	243.129	12.674	233.247	12.116	212.016
	%CV	N/A	155	67	158	56	149	41	147
	N	1	3	3	3	3	3	3	3

BLOQ – Below the limit of quantitation, < 10 ng/mL; N represents the number of rats reporting with RTD-1 concentrations above the limit of quantitation.
Values are presented as the mean ± SD of N = 3 and the average ± the range of N = 2.
N/A - not applicable.

TABLE 7

Group		Sampling Time							
		Pre-dose	Time Post Dosing Relative to Pre-dose						
			30 min	1 hr	2 hr	4 hr	8 hr	12 hr	24 hr
3-TK RTD-1 Mid Dose (2 mg/kg/dose)		**Day 1**							
	Mean	BLOQ	16.871*	15.297	12.222	13.357	17.759*	20.997	13.849
	SD	N/A	5.493	3.621	0.856	0.350	1.003	6.238	2.506
	%CV	N/A	33	24	7	3	6	30	18
	N	0	3	3	2	3	3	3	3
		Day 13							
	Mean	BLOQ	16.534	18.211	14.094	15.389	**23.916**	27.988	**37.786***
	SD	N/A	0.450	3.326	1.372	3.575	2.707	9.097	8.818
	%CV	N/A	3	18	10	23	11	33	23
	N	0	3	3	3	3	3	3	3
		Day 41							
	Mean	185.906	100.112	195.411	100.134	212.986	127.257	296.145	127.434
	SD	264.656	138.637	253.419	137.944	280.436	166.411	411.433	161.438
	%CV	142	138	130	138	132	131	139	127
	N	3	3	3	3	3	3	3	3

BLOQ – Below the limit of quantitation, < 10 ng/mL; N represents the number of rats reporting with RTD-1 concentrations above the limit of quantitation.

Values are presented as the mean ± SD of N = 3 and the average ± the range of N = 2.

For N = 3, **bold** values are significantly different from Day 1; pair-wise comparison, $p < 0.05$ Student's t-test.

N/A - not applicable.

TABLE 8

Group		Sampling Time							
		Pre-dose	Time Post Dosing Relative to Pre-dose						
			30 min	1 hr	2 hr	4 hr	8 hr	12 hr	24 hr
4-TK ORTD-1 High Dose (4 mg/kg/dose)		**Day 1**							
	Mean	BLOQ	19.827	17.019	14.163	23.085	27.873	37.129*	36.851
	SD	N/A	4.754	5.943	2.337	3.617	5.989	10.687	8.741
	%CV	N/A	24	35	16	16	21	29	24
	N	0	3	3	3	3	3	3	3
		Day 13							
	Mean	56.688	23.650*	109.634	**19.566***	106.184	45.219	177.471	51.198
	SD	66.312	2.089	135.109	1.845	131.529	14.302	207.364	21.121
	%CV	117	9	123	9	124	32	117	41
	N	3	3	3	3	3	3	3	3
		Day 41							
	Mean	171.510	234.782	225.608	194.810	210.728	214.045	305.575	263.958
	SD	120.089	319.717	159.433	270.617	150.795	267.542	263.070	352.308
	%CV	70	136	71	139	72	125	86	133
	N	3	3	3	3	3	3	3	3

BLOQ – Below the limit of quantitation, < 10 ng/mL; N represents the number of rats reporting with RTD-1 concentrations above the limit of quantitation.

Values are presented as the mean ± SD of N = 3 and the average ± the range of N = 2.

For N = 3, **bold** values are significantly different from Day 1; pair-wise comparison, $p < 0.05$ Student's t-test.

N/A - not applicable.

[0054] Table 9 shows typical results for dose proportionality in regard to C_{max} and $AUC_{0\text{-}TLast}$ for male and female rats.

TABLE 9

Dose Day	Dose Ratio Mid to Low Dose	Dose Ratio High to Low Dose	C_{max} Ratio Mid to Low Dose	C_{max} Ratio High to Low Dose	$AUC_{0\text{-}TLast}$ Ratio Mid to Low Dose	$AUC_{0\text{-}TLast}$ Ratio High to Low Dose
RTD-1, Male						
Day 1	2	4	1.78	1.90	2.01	2.84
Day 13			0.39	0.73	0.54	0.82
Day 41			1.26	2.64	1.60	3.75
RTD-1, Female						
Day 1	2	4	1.11	1.96	1.12	2.08
Day 13			0.39	1.82	0.52	1.95
Day 41			1.67	1.73	2.19	2.88

FIG. 7A provides a graph depicting results of a C_{max} vs. θ-defensin dose linearity study for male and female rats at day 1. FIG. 7B provides a graph depicting results of a C_{max} vs. θ-defensin dose linearity study for male and female rats at day 41. FIG. 8A provides a graph depicting results of an $AUC_{0\text{-}TLast}$ vs. θ-defensin dose linearity study for male and female rats at day 1. FIG. 8B provides a graph depicting results of an $AUC_{0\text{-}TLast}$ vs. θ-defensin dose linearity study for male and female rats at day 41.

Human clinical trials

[0055] The θ-defensin RTD-1 was formulated in a mildly acidic buffer containing propylene glycol as described above, and administered subcutaneously to male and female subjects ranging in age from 23 to 73 followed by determination of plasma levels of the θ-defensin. Subjects received a θ-defensin of 20 μg/kg, 40μg/kg, 80 μg/kg, 160 μg/kg, or 325 μg/kg by subcutaneous

injection. It should be appreciated that these doses are, relatively, substantially smaller than those used in the animal studies described above. Subjects receiving 20 to 80 μg/kg received doses delivered at a single site. Subjects receiving larger amounts received their doses distributed between two sites. FIG. 9 shows typical results for measurement of θ-defensin concentration (ng/mL) in plasma over time for the different treatment groups. As shown, bioavailability on subcutaneous injection is dramatically increased in humans relative to rats. Similarly improved results for human subjects were noted relative to canine and porcine animal models of subcutaneous injection with RTD-1. The dependence of C_{max} (ng/mL) on RTD-1 dose (μg/kg) is shown in FIG. 10A. Results of a similar study of dependence of $AUC_{0\text{-}TLast}$ on RTD-1 dose (μg/kg) is shown in FIG. 10B. The dependence of C_{max} (ng/mL) on total RTD-1 administered (mg) is shown in FIG. 11A. Results of a similar study of dependence of $AUC_{0\text{-}TLast}$ on total RTD-1 administered (mg) is shown in FIG. 10B. Both $AUC_{0\text{-}TLast}$ and C_{max} are approximately linear functions of the θ-defensin dose. Remarkably, far lower doses of the θ-defensin are required to reach similar plasma concentrations in humans than were noted in test animals. Inventors anticipate that similar or improved results will be observed with analogs of θ-defensin.

[0056] It should be apparent to those skilled in the art that many more modifications besides those already described are possible without departing from the inventive concepts herein. The inventive subject matter, therefore, is not to be restricted except in the spirit of the appended claims. Moreover, in interpreting both the specification and the claims, all terms should be interpreted in the broadest possible manner consistent with the context. In particular, the terms "comprises" and "comprising" should be interpreted as referring to elements, components, or steps in a non-exclusive manner, indicating that the referenced elements, components, or steps may be present, or utilized, or combined with other elements, components, or steps that are not expressly referenced. Where the specification claims refer to at least one of something selected from the group consisting of A, B, C .... and N, the text should be interpreted as requiring only one element from the group, not A plus N, or B plus N, etc.