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- (71) Applicant: **WAKE FOREST UNIVERSITY HEALTH SCIENCES** [US/US]; 575 N. Patterson Avenue, Suite 550, Winston-Salem, NC 27101 (US).
- (72) Inventor; and
- (71) Applicant : **MELENDEZ, Giselle C.** [US/US]; 810 Windalier Lane, Winston-Salem, NC 27106 (US).
- (74) Agent: **OLIVE, Bentley J.**; Olive Law Group, PLLC, 125 Edinburgh South Drive, Suite 100, Cary, NC 27511 (US).
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(54) Title: METHODS AND COMPOSITIONS FOR TREATING ANTHRACYCLINE-INDUCED CARDIOMYOPATHY USING NEUROKININ-1 RECEPTOR ANTAGONISTS

(57) Abstract: The presently disclosed subject matter provides methods and compositions for the treatment of anthracycline-induced cardiomyopathy, particularly doxorubicin- induced cardiomyopathy, using neurokinin-1 (NK-1) receptor antagonists.



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METHODS AND COMPOSITIONS FOR TREATING ANTHRACYCLINE-
INDUCED CARDIOMYOPATHY USING NEUROKININ-1 RECEPTOR
ANTAGONISTS

5 CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 61/951,605, filed March 12, 2014, which is incorporated herein by reference in its entirety.

10 FIELD OF INVENTION

The invention relates to the fields of pharmacology and medicine, and provides therapeutic methods and compositions for the treatment of anthracycline-induced cardiomyopathy, particularly doxorubicin-induced cardiomyopathy, using neurokinin-1 (NK-1) receptor antagonists.

15

BACKGROUND

Anthracycline antineoplastics such as Doxorubicin, Daunorubicin, Epirubicin, THP-Adriamycin, and Idarubicin are among the most active anticancer drugs and are effective against malignancies like leukemias, lymphomas and many solid cancers.

20 Doxorubicin is the drug of choice, alone or in combination with other chemotherapeutic agents, in the treatment of metastatic adenocarcinoma of the breast, carcinoma of the bladder, bronchogenic carcinoma, neuroblastoma, and metastatic thyroid carcinoma. Doxorubicin exerts its antitumour effects due to inhibition of DNA replication by intercalating between base pairs and/or steric inhibition of RNA
25 activity.

Cardiotoxicity is the major limitation in the use of doxorubicin (Weiss (1992) *Semin. Oncol.* 19:670-686). The risk of developing cardiomyopathy becomes unacceptably high beyond the cumulative dose of 550 mg/m² of body surface area (Lefrak et al. (1973) *Cancer* 32:302-314). In addition to clinical heart failure,
30 cardiotoxicity encompasses clinical cardiotoxicity such as congestive heart failure and/or cardiac arrhythmias, and subclinical cardiotoxicity such as that detected by pathologic changes in cardiac biopsy or decrease in ventricular ejection fractions.

It has therefore been found that doxorubicin treatment often must be terminated before the maximum effective cumulative dose has been administered to a patient bearing a neoplasm, because of the development of life-threatening cardiomyopathy. Accordingly, while doxorubicin is considered a highly effective anti-tumor agent, its effectiveness is significantly reduced by the concomitant cardiotoxicity encountered with use of the drug.

The complete mechanism for doxorubicin and other anthracycline-induced cardiotoxicity are not completely understood. Anthracycline-induced cardiomyopathy, particularly doxorubicin-induced cardiomyopathy, has been ascribed to several different mechanisms, including lipid peroxidation (Bordoni et al. (1999) *Biochim. Biophys. Acta* 1440:100-106; Young et al. (1981) *N. Engl. J. Med.* 305:139-153), free radical formation (Yin et al. (1998) *Biochem. Pharmacol.* 56:87-93; Hershko et al. (1993) *Leuk. Lymphoma* 11:207-214; Young et al. (1981) *N. Engl. J. Med.* 305:139-153), mitochondrial damage (Cini Neri et al. (1991) *Oncology* 48: 327-333), iron dependent oxidative damage to biological macromolecules (Thomas and Aust (1986) *Arch. Biochem. Biophys.* 248:684-689), and the induction of apoptosis in cardiomyocytes (Arola et al. (2000) *Cancer Res.* 60: 1789-1792). Recently, cardiomyocyte-specific deletion of *Top2b* (encoding topoisomerase-II β) has been shown to protect mice from the development of doxorubicin-induced progressive heart failure, suggesting that doxorubicin-induced cardiotoxicity may be mediated by topoisomerase-II β in cardiomyocytes (Zhang et al. (2012) *Nat. Med.* 18:1639-1642).

To date, treatments for anthracycline-induced cardiomyopathy, particularly doxorubicin-induced cardiomyopathy, have been limited. For example, in spite of purported links between doxorubicin-induced free radical formation and cardiomyopathy, several well documented free radical scavengers and/or antioxidants have not shown protective effects against doxorubicin-induced cardiotoxicity *in vivo*. For example, studies with N-acetylcysteine or Vitamin E have shown that neither compound prevented or significantly reduced cardiac damage induced by doxorubicin treatment (Herman et al. (1985) *Cancer Res.* 45:276-281; van Vleet et al. (1980) *Am. J. Pathol.* 99:13-22; Breed et al. (1980) *Cancer Res.* 40:2033-2038; Legha et al. (1982) *Ann. N.Y. Acad. Sci.* 393:411-418; Myers et al. (1983) *Semin. Oncol.* 10:53-55).

Dexrazoxane, an iron chelator, has been used clinically to treat doxorubicin-induced cardiotoxicity (Swain et al. (1997) *J. Clin. Oncol.* 15:1333-1340; Swain et al. (1997) *J. Clin. Oncol.* 15:1318-1332). However, in 2011 the U.S. Food and Drug Administration withdrew general approval for use of dexrazoxane for cardioprotection, restricting use only in adult patients with breast cancer who have received > 300 mg/m² doxorubicin. This action was based on a decision made by the European Medicines Agency to also withdraw the drug based on two clinical trials that showed a possibly higher rate of secondary malignancies and acute myelogenous leukemia in pediatric patients treated for different cancers with both dexrazoxane and other chemotherapeutic agents associated with secondary malignancies (Tebbi et al. (2007) *J. Clin. Oncol.* 25: 493–500; Salzer et al. (2010) *Leukemia* 24: 355–370; U.S. Food and Drug Administration, “FDA Statement on Dexrazoxane”, Jul. 20, 2011).

Accordingly, there remains a need for improved therapies to treat anthracycline-induced cardiomyopathy, particularly doxorubicin-induced cardiomyopathy.

SUMMARY

The presently disclosed subject matter provides methods and compositions for treating anthracycline-induced cardiomyopathy, particularly doxorubicin-induced cardiomyopathy, using neurokinin-1 (NK-1) receptor antagonists.

In some embodiments, the presently disclosed subject matter relates to a method for treating anthracycline-induced cardiomyopathy in a subject in need thereof, the method comprising administering to the subject an effective amount of a neurokinin-1 (NK-1) receptor antagonist. In one embodiment, the anthracycline and the NK-1 receptor antagonist are administered simultaneously to the subject. In another embodiment, the anthracycline and the NK-1 receptor antagonist are administered sequentially to the subject, particularly wherein the NK-1 receptor antagonist is administered prior to the anthracycline administration or wherein the NK-1 receptor antagonist is administered after the anthracycline administration, more particularly wherein the NK-1 receptor antagonist is administered for at least 2 consecutive days after treatment with the anthracycline. In another embodiment, the anthracycline is selected from the group consisting of doxorubicin, daunorubicin, epirubicin, idarubicin, and valrubicin, or a pharmaceutically acceptable salt thereof.

In a further embodiment, the NK-1 receptor antagonist is aprepitant, or a pharmaceutically acceptable salt thereof. In yet another embodiment, the method further comprises administration of an additional active agent, particularly wherein the additional active agent is selected from the group consisting of an antioxidant, an iron chelator, a statin, an endothelin-1 receptor antagonist, and an angiotensin-converting enzyme inhibitor.

In other embodiments, the presently disclosed subject matter is directed to a pharmaceutical composition comprising an anthracycline, an NK-1 receptor antagonist, and a pharmaceutically acceptable carrier. In one embodiment, the anthracycline is selected from the group consisting of doxorubicin, daunorubicin, epirubicin, idarubicin, and valrubicin, or a pharmaceutically acceptable salt thereof. In a further embodiment, the NK-1 receptor antagonist is aprepitant, or a pharmaceutically acceptable salt thereof. In yet another embodiment, the pharmaceutical composition further comprises an additional active agent, particularly wherein the additional active agent is selected from the group consisting of an antioxidant, an iron chelator, a statin, an endothelin-1 receptor antagonist, and an angiotensin-converting enzyme inhibitor.

In additional embodiments, the presently disclosed subject matter is directed to a kit for treating anthracycline-induced cardiomyopathy in a subject in need thereof, the kit comprising an NK-1 receptor antagonist and instructions for administration of the NK-1 receptor antagonist to the subject in an amount effective to treat anthracycline-induced cardiomyopathy. In one embodiment, the NK-1 receptor antagonist is aprepitant, or a pharmaceutically acceptable salt thereof. In other embodiments, the kit further comprises an anthracycline, wherein the instructions further comprise instructions for administration of the anthracycline and the NK-1 receptor antagonist to the subject. In another embodiment, the anthracycline is selected from the group consisting of doxorubicin, daunorubicin, epirubicin, idarubicin, and valrubicin, or a pharmaceutically acceptable salt thereof. In a further embodiment, the kit comprises separated dosage units, wherein at least one dosage unit comprises the anthracycline and at least one other dosage unit comprises the NK-1 receptor antagonist. In yet another embodiment, the instructions further comprise instructions for simultaneous administration of the anthracycline and the NK-1 receptor antagonist to the subject. In still another embodiment, the instructions

further comprise instructions for sequential administration of the anthracycline and the NK-1 receptor antagonist to the subject.

Certain aspects of the presently disclosed subject matter having been stated hereinabove, which are addressed in whole or in part by the presently disclosed subject matter, other aspects will become evident as the description proceeds when taken in connection with the accompanying Examples and Figures as best described herein below.

BRIEF DESCRIPTION OF THE FIGURES

Having thus described the presently disclosed subject matter in general terms, reference will now be made to the accompanying Figures, which are not necessarily drawn to scale, and wherein:

FIG. 1 shows Echocardiographic parameters in WT sham ($n = 8$), WT Fistula ($n = 7$), $TAC1^{-/-}$ Sham ($n = 7$), and $TAC1^{-/-}$ Fistula ($n = 7$) at 0, 14 and 28 days post fistula. (A) Echocardiographic-derived heart rate, (B) left ventricular internal diameter in diastole (LVIDd), (C) Per cent change in left ventricular internal diameter in diastole, (D) Left ventricle posterior wall thickness in diastole (LVPWd), (E) Per cent change of left ventricle posterior wall thickness in diastole, and (F) Fractional shortening at 28 days post fistula. All values are mean $\pm$ SEM. $*P < 0.05$ vs. WT sham and $\dagger P < 0.05$ vs. WT fist.;

FIG. 2 shows (A) Myocardial levels of $TNF-\alpha$, (B) myocardial matrix metalloproteinase (MMP) activity, and (C) number of TUNEL⁺ nuclei per square millimetre of left ventricular section in WT sham ($n = 8$), WT fistula ($n = 7$), $TAC1^{-/-}$ sham ($n = 7$), and $TAC1^{-/-}$ fistula ($n = 7$). All values are mean $\pm$ SEM. $*P < 0.05$ vs. WT sham and $\dagger P < 0.05$ vs. WT fist.;

FIG. 3 shows (A) Graphical representation of collagen volume fraction and (B) representative images of picrosirius red stained sections taken at 20 $\times$ magnification from WT sham ($n = 8$), WT fistula ($n = 7$), $TAC1^{-/-}$ sham ($n = 7$), and $TAC1^{-/-}$ fistula ($n = 7$) after 28 days post-surgery. All values are mean $\pm$ SEM. $*P < 0.05$ vs. WT sham;

FIG. 4 shows (A) Concentration–response curves for histamine release in response to substance P ($n = 5$) and NKA ($n = 4$) ($*P < 0.05$ vs. NKA), (B) Histamine release from isolated cardiac mast cells that were untreated (control), substance P-

treated (100 μ M), pre-treated with L 732 198 (20 μ M) prior to stimulation with substance P, or pre-treated with GR 159 897 (10 μ M) prior to stimulation with substance P (* P < 0.05 vs. cont; † P < 0.05 vs. substance P), and (C) TNF- α release from a mixed population of isolated cardiac inflammatory cells in response to substance P (100 μ M). All values are mean $\pm$ SEM (* P < 0.05 vs. control);

FIG. 5 shows (A) Graphic representation of LV collagen volume fraction (n = 5). Values are mean $\pm$ SEM, (B) representative images from sham, untreated fistula, and fistula treated with the NK-1 receptor antagonist, L 732 138, (C) changes in cardiac mast cell density for sham, untreated fistula, and fistula treated with the NK-1 receptor antagonist, L 732 138 (n = 5). Values are mean $\pm$ SD, and (D) myocardial TNF- α levels following treatment with the selective NK-1 receptor antagonist, L 732 138 (5 mg/kg/day) (n = 5). Values are mean $\pm$ SEM. * P < 0.05 vs. sham; † P < 0.05 vs. untreated fist; and

FIG. 6 shows a schematic depicting the proposed mechanism by which sensory nerves initiate adverse myocardial remodelling. Substance P activates cardiac mast cells via the neurokinin (NK)-1 receptor. Mast cells induce matrix metalloproteinase (MMP) activation possibly via release of TNF- α . MMP activation results in adverse myocardial remodelling and ultimately heart failure.

DETAILED DESCRIPTION

The presently disclosed subject matter now will be described more fully hereinafter with reference to the accompanying Figures, in which some, but not all embodiments of the presently disclosed subject matter are shown. Like numbers refer to like elements throughout. The presently disclosed subject matter may be embodied in many different forms and should not be construed as limited to the embodiments set forth herein; rather, these embodiments are provided so that this disclosure will satisfy applicable legal requirements. Indeed, many modifications and other embodiments of the presently disclosed subject matter set forth herein will come to mind to one skilled in the art to which the presently disclosed subject matter pertains having the benefit of the teachings presented in the foregoing descriptions and the associated Figures. Therefore, it is to be understood that the presently disclosed subject matter is not to be limited to the specific embodiments disclosed and that

modifications and other embodiments are intended to be included within the scope of the appended claims.

The presently disclosed subject matter is directed to methods and compositions for the treatment of anthracycline-induced cardiomyopathy, particularly doxorubicin-induced cardiomyopathy, using neurokinin-1 (NK-1) receptor antagonists. As described more fully below, the presently disclosed subject matter relates to the finding that inhibiting the interaction of the NK-1 receptor with its ligands (such as its endogenous ligand Substance P) reduces or eliminates anthracycline-induced cardiomyopathy, particularly doxorubicin-induced cardiomyopathy.

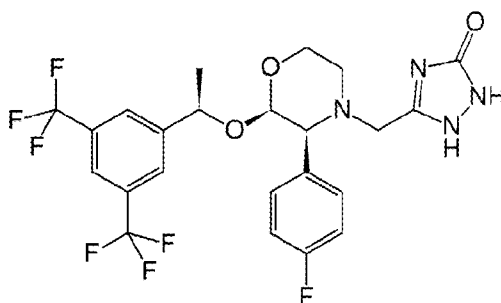
I. METHODS OF TREATMENT

Substance P is a naturally occurring undecapeptide belonging to the tachykinin family of peptides, the latter being so-named because of their prompt contractile action on extravascular smooth muscle tissue. Tachykinins are distinguished by a conserved carboxyl-terminal sequence. In addition to Substance P, known mammalian tachykinins include neurokinin A and neurokinin B. However, current nomenclature designates the receptors for Substance P, neurokinin A, and neurokinin B as neurokinin-1 (NK-1), neurokinin-2 (NK-2), and neurokinin-3 (NK-3), respectively.

In one embodiment, the presently disclosed subject matter is directed to methods for the treatment of anthracycline-induced cardiomyopathy, particularly doxorubicin-induced cardiomyopathy, comprising administering to a subject in need thereof a therapeutically effective amount of an NK-1 receptor antagonist. NK-1 receptor antagonists of use in this method are fully described, for example, in U.S. Patent Nos. 5,162,339, 5,232,929, 5,242,930, 5,373,003, 5,387,595, 5,459,270, 5,494,926, 5,496,833, 5,637,699, 5,719,147; European Patent Publication Nos. EP 0 360 390, 0 394 989, 0 428 434, 0 429 366, 0 430 771, 0 436 334, 0 443 132, 0 482 539, 0 498 069, 0 499 313, 0 512 901, 0 512 902, 0 514 273, 0 514 274, 0 514 275, 0 514 276, 0 515 681, 0 517 589, 0 520 555, 0 522 808, 0 528 495, 0 532 456, 0 533 280, 0 536 817, 0 545 478, 0 558 156, 0 577 394, 0 585 913, 0 590 152, 0 599 538, 0 610 793, 0 634 402, 0 686 629, 0 693 489, 0 694 535, 0 699 655, 0 699 674, 0 707 006, 0 708 101, 0 709 375, 0 709 376, 0 714 891, 0 723 959, 0 733 632 and 0 776

893; PCT International Patent Publication Nos. WO 90/05525, 90/05729, 91/09844, 91118899, 92/01688, 92/06079, 92/12151, 92/15585, 92/17449, 92/20661, 92/20676, 92/21677, 92/22569, 93/00330, 93/00331, 93/01159, 93/01165, 93/01169, 93/01170, 93/06099, 93/09116, 93/10073, 93/14084, 93/14113, 93/18023, 93/19064, 93/21155, 93/21181, 93/23380, 93/24465, 94/00440, 94/01402, 94/02461, 94/02595, 94/03429, 94/03445, 94/04494, 94/04496, 94/05625, 94/07843, 94/08997, 94/10165, 94/10167, 94/10168, 94/10170, 94/11368, 94/13639, 94/13663, 94/14767, 94/15903, 94/19320, 94/19323, 94/20500, 94/26735, 94/26740, 94/29309, 95/02595, 95/04040, 95/04042, 95/06645, 95/07886, 95/07908, 95/08549, 95/11880, 95/14017, 95/15311, 95/16679, 95/17382, 95/18124, 95/18129, 95/19344, 95/20575, 95/21819, 95/22525, 95/23798, 95/26338, 95/28418, 95/30674, 95/30687, 95/33744, 96/05181, 96/05193, 96/05203, 96/06094, 96/07649, 96/10562, 96/16939, 96/18643, 96/20197, 96/21661, 96/29304, 96/29317, 96/29326, 96/29328, 96/31214, 96/32385, 96/37489, 97/01553, 97/01554, 97/03066, 97/08144, 97/14671, 97/17362, 97/18206, 97/19084, 97/19942 and 97/21702; and in British Patent Publication Nos. 2 266 529, 2 268 931, 2 269 170, 2 269 590, 2 271 774, 2 292 144, 2 293 168, 2 293 169, and 2 302 689. The preparation of such compounds is fully described in the aforementioned patents and publications, which are incorporated herein by reference.

In a particular embodiment, the NK-1 receptor antagonist for use in presently disclosed methods is aprepitant, a chemical compound selected from: 2-(R)-(1-(R)-(3,5-bis(trifluoromethyl)phenyl)ethoxy)-3-(S)-(4-fluorophenyl)-4-(3-(5-oxo-1H,4H-1,2,4-thiazolo)methyl)morpholine or a pharmaceutically acceptable salt thereof, and that is structurally represented by Formula (I):



Formula (I)

Aprepitant is useful in the treatment of emesis concomitant with cancer chemotherapy and is manufactured and commercially sold in the market by Merck

and Co. under the brand name EMEND™. U.S. Patent No. 5,719,147 discloses the preparation of aprepitant and its pharmaceutically acceptable salts, their pharmaceutical compositions and methods of use. U.S. Patent Nos. 6,096,742 and 6,583,142 disclose crystalline Form I and Form II of aprepitant, processes for making these forms, pharmaceutical compositions comprising them, and methods of use. These patents specifically claim aprepitant Form I substantially free from Form II, and aprepitant Form II substantially free from Form I. Various formulations and polymorphic forms of aprepitant have also been described (U.S. Patent Nos. 8,217,039 and 8,258,132).

10 In some embodiments, the process for the preparation of crystalline Form I of aprepitant involves crystallizing aprepitant from ethanol, 2-propanol, acetonitrile and isopropyl acetate. In other embodiments, Form I is prepared by heating a sample of aprepitant Form II to a temperature range of 215°C to 230°C and cooling to ambient conditions. In still other embodiments, Form I of aprepitant is prepared on a larger scale by suspending aprepitant in a solution of methanol/water in the ratio of 2:1 (v/v), adding seed crystals of Form I and stirring the resultant mixture at about 0°C to 50°C for a period sufficient to result in the formation of Form I.

In some embodiments, the process for the preparation of crystalline Form II of aprepitant is disclosed in Example 75 of U.S. Patent No. 5,719,147.

20 In some embodiments, crystalline Form I of aprepitant is reported to have superior properties over other forms of aprepitant, i.e., Form II, in that this form demonstrates superior thermodynamic stability and is non-hygroscopic when compared with other crystalline forms of aprepitant. In other embodiments, crystalline Form I of aprepitant has been shown to have a lower solubility (0.9 ± 0.1 mg/ml) when compared with Form II (1.3 ± 0.2 mg/ml) in a 2:1 v/v methanol/water mixture at 0° C. The numeric ratio of these solubilities (1.4) can indicate a higher stability of Form I over Form II.

30 Different morphological forms of the same compound, such as Form I and Form II of aprepitant, may exhibit significantly different properties such as, for example, enhanced thermodynamic stability or improved dissolution characteristics among other properties. In some embodiments, improved properties help in developing pharmaceutical dosage forms with improved stability or handling characteristics. Accordingly, in some embodiments, a single polymorphic form

substantially free from other polymorphic forms or that a mixture of different polymorphic forms in specified ratios are used in the preparation of a pharmaceutical formulation. In other embodiments, a mixture of polymorphic forms can provide a viable alternative in the development of a formulation of aprepitant with improved properties.

Accordingly, in some embodiments, the presently disclosed subject matter provides a method for treating anthracycline-induced cardiomyopathy, particularly doxorubicin-induced cardiomyopathy, in a subject in need thereof, the method comprising administering to the subject an effective amount of a neurokinin-1 (NK-1) receptor antagonist. In some embodiments, the NK-1 receptor antagonist is aprepitant. The term "receptor antagonist" as used herein refers to a type of receptor ligand or drug that does not provoke a biological response itself upon binding to a receptor, but blocks or dampens agonist-mediated responses. An agonist is a molecule that binds to a receptor and activates the receptor to produce a biological response.

In another embodiment, the presently disclosed subject matter is directed to methods for the treatment of anthracycline-induced cytotoxicity, particularly doxorubicin-induced cardiomyopathy, in a plurality of mammalian cardiac cells in a subject undergoing treatment with an anthracycline, comprising administering to the subject a therapeutically effective dose of an NK-1 receptor antagonist.

Cardiomyopathy is well-known to result from the cardiotoxicity of anthracyclins such as doxorubicin in patients who receive a cumulative dose of more than about 500 mg/m². Although several mechanisms, such as free radical-dependent lipid peroxidation, mitochondrial impairment, and modification of cardiac calcium transport, have been reported to be the cause of the cardiotoxicity of anthracyclines, the precise mechanism of myocardial impairment remains unclear.

When cardiomyopathy is sufficiently advanced, it causes congestive heart failure, with physiological symptoms including breathlessness with exertion or even at rest, swelling of the legs, ankles and feet, bloating (distention) of the abdomen with fluid, fatigue, irregular heartbeats, and dizziness, lightheadedness and fainting.

Anthracycline-induced cardiomyopathy, particularly doxorubicin-induced cardiomyopathy, may be divided into acute, subacute, and late forms (Bristow et al. (1978) *Cancer Treat. Rep.* 62:873-879). The acute form is myocarditis/pericarditis

syndrome that starts within 24 hours of the infusion and is not associated with poor long-term prognosis. Subacute toxicity ensues weeks after doxorubicin treatment but may be seen as late as 30 months. This form is associated with chronic changes and mortality may be as high as 60% (Goorin et al. (1981) *Cancer* 47:2810-2816).

5 Chronic toxicity may be evident as late as 4 to 20 years after the treatment with doxorubicin and it is accompanied by clinical heart failure and echocardiographic and pathologic changes. In some embodiments, the presently disclosed methods are used to treat acute, subacute, or late forms of anthracycline-induced cardiomyopathy, particularly doxorubicin-induced cardiomyopathy.

10 An indicator of anthracycline-induced cardiomyopathy, particularly doxorubicin-induced cardiomyopathy, is increased cardiomyocyte apoptosis (Arola et al. (2000) *Cancer Res.* 60:1789-1702). Cardiomyopathy is also accompanied by an increase in fibrosis of the cardiac tissue. Fibrosis may be measured using Picro-Sirius Red staining, a method well-known to one skilled in the art.

15 Assays to determine whether or not an NK-1 receptor antagonist is effective in preventing the onset of cardiomyopathy, or reducing its progression, are known to persons having ordinary skill in the art and include monitoring cardiac function by measuring fractional shortening, ejection fraction, end-diastolic volume (methods described in Bielecka-Dabrowa et al. (2008) *Cardiology J.* 278:1-5; Nellessen et al. (2006) *Clin. Cardiol.* 29:219-224).

Progression of cardiomyopathy may be monitored in part by measuring levels of serum biomarkers, such as creatine kinase, troponin, or brain natriuretic peptide (BNP).

Progression of cardiomyopathy may be assessed in part by measuring
 25 fractional shortening (FS) or ejection fraction (EF). FS is used to measure left ventricle performance by measuring the change in the diameter of the left ventricle between the contracted and relaxed state on M-mode tracings and calculating the ratio according to the formula: $[(LV \text{ end-diastolic diameter} - LV \text{ end-systolic diameter}) / LV \text{ end-diastolic diameter}] \times 100$. EF is calculated from left ventricular volumes
 30 determined by 2-dimensional echo, as $[(LV \text{ end-diastolic volume} - LV \text{ end-systolic volume}) / LV \text{ end-diastolic volume}] \times 100$. A decrease in FS or EF is indicative of heart damage due to cardiotoxicity.

In one embodiment, a therapeutically effective amount of an NK-1 receptor antagonist is administered to a subject undergoing doxorubicin treatment, wherein the an NK-1 receptor antagonist prevents more than 10-20% reduction in the FS or EF as compared to a subject undergoing doxorubicin treatment but is not administered an NK-1 receptor antagonist. In another embodiment, administration of an NK-1 receptor antagonist to a subject undergoing doxorubicin treatment prevents more than 5% reduction in the FS or EF as compared to a subject undergoing doxorubicin treatment but is not administered an NK-1 receptor antagonist.

In one embodiment, a therapeutically effective amount of an NK-1 receptor antagonist is administered to a subject undergoing doxorubicin treatment, wherein the an NK-1 receptor antagonist prevents more than 10-20% increase in the end-diastolic volume as compared to a subject undergoing doxorubicin treatment but is not administered an NK-1 receptor antagonist. In another embodiment, administration of an NK-1 receptor antagonist to a subject undergoing doxorubicin treatment prevents more than 5% increase in the end-diastolic volume as compared to a subject undergoing doxorubicin treatment but is not administered an NK-1 receptor antagonist.

In one embodiment, the method prevents a decrease in fractional shortening in the subject by more than 5%, 8%, 10%, 12%, 15% or 20% as compared to fractional shortening in a subject undergoing anthracycline treatment but not administered an NK-1 receptor antagonist.

In one embodiment, the method prevents an increase in the amount of creatine kinase or troponin in the serum of the subject by more than 2-fold, 4-fold, or 5-fold as compared to the amount of creatine kinase or troponin in the serum of the subject undergoing anthracycline treatment but not administered an NK-1 receptor antagonist.

In one embodiment, the method prevents an increase in the percentage of cardiac fibrosis area by more than 4%, 6%, 8%, 10% or 12% in the heart of the subject undergoing the anthracycline treatment but not administered an NK-1 receptor antagonist.

By “administered with” or “in combination with” is meant the administration of multiple therapeutic agents either simultaneously, sequentially, or a combination thereof. Therefore, a subject administered a combination of therapeutic agents, can receive an anthracycline and a NK-1 receptor antagonist as described herein at the

same time (i.e., simultaneously) or at different times (i.e., sequentially, in either order, on the same day or on different days), so long as the effect of the combination of both agents is achieved in the subject. When administered sequentially, the agents can be administered within 1, 5, 10, 30, 60, 120, 180, 240 minutes or longer of one another.

- 5 In other embodiments, agents administered sequentially, can be administered within 1, 5, 10, 15, 20 or more days of one another.

In some embodiments, the anthracycline and the NK-1 receptor antagonist are administered simultaneously to the subject. In other embodiments, the anthracycline and the NK-1 receptor antagonist are administered sequentially to the subject.

- 10 Accordingly, in one embodiment, the dose of an NK-1 receptor antagonist is co-administered with the anthracycline to the subject. When administered sequentially, the agents can be administered within 1, 5, 10, 30, 60, 120, 180, 240 minutes or longer of one another. In other embodiments, agents administered sequentially, can be administered within 1, 5, 10, 15, 20 or more days of one another.

- 15 Agents administered simultaneously may be administered to the subject as separate pharmaceutical compositions, or may be administered to a subject as a single pharmaceutical composition comprising multiple agents.

- In one embodiment, the dose of an NK-1 receptor antagonist and an anthracycline are administered to the subject on the same day. In another
20 embodiment, the dose of an NK-1 receptor antagonist is administered to the subject after administration of the anthracycline. In another embodiment, the dose of an NK-1 receptor antagonist is administered for 1, 2, 3, 4, 5, 6, 7, 8 or more consecutive days following the anthracycline administration. In yet another embodiment, the dose of an NK-1 receptor antagonist is administered daily for a period of at least 1, 2, 3, 4, 5,
25 6, or 7 or more days.

- In another embodiment, the dose of an NK-1 receptor antagonist is administered to the subject prior to administration of the anthracycline. In another embodiment, the dose of an NK-1 receptor antagonist is administered to the subject 1 or 2 days prior to administration of the anthracycline. In yet another embodiment, the
30 dose of an NK-1 receptor antagonist is administered at least 1, 2, 3, 4, 5, 6 hours prior to administration of the anthracycline.

In some embodiments, the NK-1 receptor antagonist is administered with an additional active agent. In other embodiments, the additional active agent is selected

from the group consisting of consisting of an antioxidant, an iron chelator, a statin, an endothelin-1 receptor antagonist, and an angiotensin-converting enzyme inhibitor, as described more fully elsewhere herein.

As used herein, the term “subject” treated by the presently disclosed methods
5 in their many embodiments is desirably a human subject, although it is to be understood that the methods described herein are effective with respect to all vertebrate species, which are intended to be included in the term “subject.” Accordingly, a “subject” can include a human subject for medical purposes, such as for the diagnosis or treatment of an existing disease, disorder, condition or the
10 prophylactic diagnosis or treatment for preventing the onset of a disease, disorder, or condition or an animal subject for medical, veterinary purposes, or developmental purposes. Suitable animal subjects include mammals including, but not limited to, primates, e.g., humans, monkeys, apes, gibbons, chimpanzees, orangutans, macaques and the like; bovines, e.g., cattle, oxen, and the like; ovines, e.g., sheep and the like;
15 caprines, e.g., goats and the like; porcines, e.g., pigs, hogs, and the like; equines, e.g., horses, donkeys, zebras, and the like; felines, including wild and domestic cats; canines, including dogs; lagomorphs, including rabbits, hares, and the like; and rodents, including mice, rats, guinea pigs, and the like. An animal may be a transgenic animal. In some embodiments, the subject is a human including, but not
20 limited to, fetal, neonatal, infant, juvenile, and adult subjects. Further, a “subject” can include a patient afflicted with or suspected of being afflicted with a disease, disorder, or condition. Thus, the terms “subject” and “patient” are used interchangeably herein. Subjects also include animal disease models (e.g., rats or mice used in experiments, and the like).

As described herein, the presently disclosed NK-1 receptor antagonists can be
25 administered to a subject for therapy by any suitable route of administration, including orally, nasally, transmucosally, ocularly, rectally, intravaginally, parenterally, including intramuscular, subcutaneous, intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, intra-articular, intra-sternal, intra-
30 synovial, intra-hepatic, intralesional, intracranial, intraperitoneal, intranasal, or intraocular injections, intracisternally, topically, as by powders, ointments or drops (including eyedrops), including buccally and sublingually, transdermally, through an inhalation spray, or other modes of delivery known in the art.

The phrases “systemic administration,” “administered systemically,” “peripheral administration” and “administered peripherally” as used herein mean the administration of the presently disclosed compositions comprising a NK-1 receptor antagonist, a compound, drug or other material other than directly into the central nervous system, such that it enters the patient’s system and, thus, is subject to metabolism and other like processes, for example, subcutaneous administration.

The phrases “parenteral administration” and “administered parenterally” as used herein mean modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intarterial, intrathecal, intracapsular, intraorbital, intraocular, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal and intrasternal injection and infusion.

The presently disclosed pharmaceutical compositions comprising a NK-1 receptor antagonist can be manufactured in a manner known in the art, e.g. by means of conventional mixing, dissolving, granulating, dragee-making, levitating, emulsifying, encapsulating, entrapping or lyophilizing processes.

Pharmaceutical compositions for oral use can be obtained through combination of the presently disclosed compositions comprising a NK-1 receptor antagonist with a solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients include, but are not limited to, carbohydrate or protein fillers, such as sugars, including lactose, sucrose, mannitol, or sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose, such as methyl cellulose, hydroxypropylmethyl-cellulose, or sodium carboxymethyl cellulose; and gums including arabic and tragacanth; and proteins, such as gelatin and collagen; and polyvinylpyrrolidone (PVP:povidone). If desired, disintegrating or solubilizing agents, such as cross-linked polyvinyl pyrrolidone, agar, alginic acid, or a salt thereof, such as sodium alginate, also can be added to the compositions.

Dragee cores are provided with suitable coatings, such as concentrated sugar solutions, which also can contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol (PEG), and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments can be added to

the tablets or dragee coatings for product identification or to characterize the quantity of compositions comprising a NK-1 receptor antagonist, e.g., dosage, or different combinations of doses.

Pharmaceutical compositions suitable for oral administration include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating, e.g., a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain active ingredients admixed with a filler or binder, such as lactose or starches, lubricants, such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, compositions comprising a NK-1 receptor antagonist can be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols (PEGs), with or without stabilizers. Stabilizers can be added as warranted. In some embodiments, the presently disclosed pharmaceutical compositions can be administered by rechargeable or biodegradable devices. For example, a variety of slow-release polymeric devices have been developed and tested *in vivo* for the controlled delivery of drugs, including proteinacious biopharmaceuticals. Suitable examples of sustained release preparations include semipermeable polymer matrices in the form of shaped articles, e.g., films or microcapsules. Sustained release matrices include polyesters, hydrogels, polylactides (U.S. Patent No. 3,773,919; EP 58,481), copolymers of L-glutamic acid and gamma ethyl-L-glutamate (Sidman et al., *Biopolymers* 22:547, 1983), poly (2-hydroxyethyl-methacrylate) (Langer et al., *J. Biomed. Mater. Res.* 15:167, 1981; Langer, *Chem. Tech.* 12:98, 1982), ethylene vinyl acetate (Langer et al., *Id.*), or poly-D-(-)-3-hydroxybutyric acid (EP 133,988A). Sustained release compositions also include liposomally entrapped compositions comprising a NK-1 receptor antagonist which can be prepared by methods known per se (Epstein et al., *Proc. Natl. Acad. Sci. U.S.A.* 82:3688, 1985; Hwang et al., *Proc. Natl. Acad. Sci. U.S.A.* 77:4030, 1980; U.S. Patent Nos. 4,485,045 and 4,544,545; and EP 102,324A). Ordinarily, the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mol % cholesterol, the selected proportion being adjusted for the optimal therapy. Such materials can comprise an implant, for example, for sustained release of the presently disclosed compositions comprising a NK-1 receptor antagonist, which, in some embodiments, can be implanted at a particular, pre-determined target site.

Pharmaceutical compositions for parenteral administration include aqueous solutions of compositions comprising a NK-1 receptor antagonist. For injection, the presently disclosed pharmaceutical compositions can be formulated in aqueous solutions, for example, in some embodiments, in physiologically compatible buffers, such as Hank's solution, Ringer's solution, or physiologically buffered saline. Aqueous injection suspensions can contain substances that increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Additionally, suspensions of compositions comprising a NK-1 receptor antagonist or vehicles include fatty oils, such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Optionally, the suspension also can contain suitable stabilizers or agents that increase the solubility of the compositions comprising a NK-1 receptor antagonist to allow for the preparation of highly concentrated solutions.

For nasal or transmucosal administration generally, penetrants appropriate to the particular barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

For inhalation delivery, the agents of the disclosure also can be formulated by methods known to those of skill in the art, and may include, for example, but not limited to, examples of solubilizing, diluting, or dispersing substances such as, saline, preservatives, such as benzyl alcohol, absorption promoters, and fluorocarbons.

Additional ingredients can be added to compositions for topical administration, as long as such ingredients are pharmaceutically acceptable and not deleterious to the epithelial cells or their function. Further, such additional ingredients should not adversely affect the epithelial penetration efficiency of the composition, and should not cause deterioration in the stability of the composition. For example, fragrances, opacifiers, antioxidants, gelling agents, stabilizers, surfactants, emollients, coloring agents, preservatives, buffering agents, and the like can be present. The pH of the presently disclosed topical composition can be adjusted to a physiologically acceptable range of from about 6.0 to about 9.0 by adding buffering agents thereto such that the composition is physiologically compatible with a subject's skin.

Regardless of the route of administration selected, the presently disclosed compositions comprising a NK-1 receptor antagonist are formulated into

pharmaceutically acceptable dosage forms such as described herein or by other conventional methods known to those of skill in the art.

The term “effective amount,” as in “a therapeutically effective amount,” of a therapeutic agent refers to the amount of the agent necessary to elicit the desired biological response. As will be appreciated by those of ordinary skill in this art, the effective amount of an agent may vary depending on such factors as the desired biological endpoint, the agent to be delivered, the composition of the pharmaceutical composition, the target tissue or cell, and the like. More particularly, the term “effective amount” refers to an amount sufficient to produce the desired effect, e.g., to reduce or ameliorate the severity, duration, progression, or onset of a disease, disorder, or condition (e.g., a disease, condition, or disorder related to anthracycline-induced cardiomyopathy, particularly doxorubicin-induced cardiomyopathy), or one or more symptoms thereof; prevent the advancement of a disease, disorder, or condition, cause the regression of a disease, disorder, or condition; prevent the recurrence, development, onset or progression of a symptom associated with a disease, disorder, or condition, or enhance or improve the prophylactic or therapeutic effect(s) of another therapy.

Actual dosage levels of the active ingredients in the presently disclosed compositions can be varied so as to obtain an amount of the active ingredient that is effective to achieve the desired therapeutic response for a particular subject, composition, route of administration, and disease, disorder, or condition without being toxic to the subject. The selected dosage level will depend on a variety of factors including the activity of the particular composition employed, the route of administration, the time of administration, the rate of excretion of the particular composition being employed, the duration of the treatment, other drugs, and/or materials used in combination with the particular composition employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

A physician having ordinary skill in the art can readily determine and prescribe the effective amount of the presently disclosed composition required. Accordingly, the dosage range for administration will be adjusted by the physician as necessary, as described more fully elsewhere herein.

II. COMPOSITIONS

In some embodiments, the presently disclosed subject matter also provides a pharmaceutical composition comprising an anthracycline, an NK-1 receptor antagonist, and a pharmaceutically acceptable carrier. In some embodiments, the anthracycline is selected from the group consisting of doxorubicin (CAS No. 23214-92-8), doxorubicin hydrochloride (CAS No. 25316-40-9), daunorubicin (CAS No. 20830-81-3), daunorubicin hydrochloride (CAS No. 23541-50-6), epirubicin (CAS No. 56420-45-2), epirubicin hydrochloride (CAS No. 56390-09-1), idarubicin (CAS No. 58957-92-9), idarubicin hydrochloride (CAS No. 57852-57-0), and valrubicin (CAS No. 56124-62-0), or pharmaceutically acceptable salts thereof. In other embodiments, the NK-1 receptor antagonist is aprepitant or pharmaceutically acceptable salts thereof.

As used herein, the term “pharmaceutically acceptable salt,” refers to salts of a free acid or a free base which are not biologically undesirable and are generally prepared by reacting the free base with a suitable organic or inorganic acid or by reacting the acid with a suitable organic or inorganic base. The term may be used in reference to any compound of the present invention. Representative salts include the following salts: Acetate, Benzenesulfonate, Benzoate, Bicarbonate, Bisulfate, Bitartrate, Borate, Bromide, Calcium Edetate, Camsylate, Carbonate, Chloride, Clavulanate, Citrate, Dihydrochloride, Edetate, Edisylate, Estolate, Esylate, Fumarate, Gluceptate, Gluconate, Glutamate, Glycolylarsanilate, Hexylresorcinate, Hydrabamine, Hydrobromide, Hydrochloride, Hydroxynaphthoate, Iodide, Isethionate, Lactate, Lactobionate, Laurate, Malate, Maleate, Mandelate, Mesylate, Methylbromide, Methylnitrate, Methylsulfate, Monopotassium Maleate, Mucate, Napsylate, Nitrate, N-methylglucamine, Oxalate, Pamoate (Embonate), Palmitate, Pantothenate, Phosphate/diphosphate, Polygalacturonate, Potassium, Salicylate, Sodium, Stearate, Subacetate, Succinate, Tannate, Tartrate, Teoclate, Tosylate, Triethiodide, Trimethylammonium and Valerate. When an acidic substituent is present, such as -COOH, there can be formed the ammonium, morpholinium, sodium, potassium, barium, calcium salt, and the like, for use as the dosage form. When a basic group is present, such as amino or a basic heteroaryl radical, such as pyridyl, an acidic salt, such as hydrochloride, hydrobromide, phosphate, sulfate, trifluoroacetate, trichloroacetate, acetate, oxalate, maleate, pyruvate, malonate, succinate, citrate,

tartarate, fumarate, mandelate, benzoate, cinnamate, methanesulfonate, ethanesulfonate, picrate and the like, and include acids related to the pharmaceutically-acceptable salts listed in Stephen M. Berge, et al., *Journal of Pharmaceutical Science*, Vol. 66(1), pp. 1-19 (1977).

5 Accordingly, the presently disclosed compositions and formulations include compositions comprising a NK-1 receptor antagonist alone or in combination with an anthracycline, in admixture with a physiologically compatible carrier or a pharmaceutically acceptable carrier, which can be administered to a subject, for example, a human subject, for therapeutic or prophylactic treatment. As used herein,
10 “pharmaceutically acceptable carrier” or “physiologically compatible carrier” refers to a physiologically acceptable diluent including, but not limited to water, phosphate buffered saline, or saline, and, in some embodiments, include an adjuvant.

 Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and can include buffers such as phosphate,
15 citrate, and other organic acids; antioxidants including ascorbic acid, BHA, and BHT; low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin or immunoglobulins; hydrophilic polymers, such as polyvinylpyrrolidone, amino acids such as glycine, glutamine, asparagine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose,
20 mannose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counter-ions such as sodium; and/or nonionic surfactants such as Tween, Pluronics, or PEG.

 In other embodiments, the pharmaceutical composition can be a lyophilized powder, optionally including additives, such as 1 mM-50 mM histidine, 0.1%-2%
25 sucrose, 2%-7% mannitol at a pH range of 4.5 to 5.5 that is combined with buffer prior to use.

 The inert ingredients and manner of formulation of the pharmaceutical compositions of the invention are conventional. Known methods of formulation used in pharmaceutical science may be followed. All of the usual types of compositions
30 are contemplated including, but not limited to, tablets, chewable tablets, capsules, and solutions. The amount of each active agent, however, is best defined as the effective amount, that is, the amount of each active agent which provides the desired dose to the subject in need of such treatment. The activity of each active agent does not

depend on the nature of the composition, so the compositions may be chosen and formulated solely for convenience and economy. Any of the active agents as described herein may be formulated in any desired form of composition.

Capsules may be prepared by mixing each active agent with a suitable diluent and filling the proper amount of the mixture in capsules. The usual diluents include inert powdered substances such as starch of many different kinds, powdered cellulose, especially crystalline and microcrystalline cellulose, sugars such as fructose, mannitol and sucrose, grain flours and similar edible powders.

Tablets may be prepared by direct compression, by wet granulation, or by dry granulation. Their formulations usually incorporate diluents, binders, lubricants and disintegrators as well as each active agent. Typical diluents include, for example, various types of starch, lactose, mannitol, kaolin, calcium phosphate or sulfate, inorganic salts such as sodium chloride and powdered sugar. Powdered cellulose derivatives are also useful. Typical tablet binders are substances such as starch, gelatin and sugars such as lactose, fructose, glucose and the like. Natural and synthetic gums are also convenient, including acacia, alginates, methylcellulose, polyvinylpyrrolidone and the like. Polyethylene glycol, ethylcellulose and waxes can also serve as binders.

A lubricant in a tablet formulation may help prevent the tablet and punches from sticking in the die. A lubricant can be chosen from such solids as talc, magnesium and calcium stearate, stearic acid and hydrogenated vegetable oils.

Tablet disintegrators are substances which swell when wetted to break up the tablet and release the compound. They include starches, clays, celluloses, alginates and gums. More particularly, corn and potato starches, methylcellulose, agar, bentonite, wood cellulose, powdered natural sponge, cation-exchange resins, alginic acid, guar gum, citrus pulp and carboxymethylcellulose, for example, may be used, as well as sodium lauryl sulfate.

Enteric formulations are often used to protect an active ingredient from the strongly acid contents of the stomach. Such formulations are created by coating a solid dosage form with a film of a polymer which is insoluble in acid environments, and soluble in basic environments. Exemplary films are cellulose acetate phthalate, polyvinyl acetate phthalate, hydroxypropyl methylcellulose phthalate and hydroxypropyl methylcellulose acetate succinate.

Tablets are often coated with sugar as a flavor and sealant. Each active agent may also be formulated as chewable tablets, by using large amounts of pleasant-tasting substances

such as mannitol in the formulation, as is now well-established practice.

- 5 Transdermal patches may be used. Typically, a patch comprises a resinous composition in which the active compound(s) will dissolve, or partially dissolve, which is held in contact with the skin by a film which protects the composition. Other, more complicated patch compositions are also in use, particularly those having a membrane pierced with innumerable pores through which the drugs are pumped by
10 osmotic action.

- In any embodiment where an active agent is included in a pharmaceutical composition, such pharmaceutical compositions may be in a form suitable for oral use, for example, as tablets, troches, lozenges, aqueous, or oily suspensions, dispersible powders or granules, emulsions, hard or soft capsules, or
15 syrups or elixirs. Compositions intended for oral use may be prepared according to any known method, and such compositions may contain one or more agents selected from the group consisting of sweetening agents, flavoring agents, coloring agents, and preserving agents in order to provide pharmaceutically elegant and palatable preparations. Tablets may contain the active ingredient in admixture with non-toxic
20 pharmaceutically-acceptable excipients which are suitable for the manufacture of tablets. These excipients may be for example, inert diluents, such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, for example corn starch or alginic acid; binding agents, for example, starch, gelatin or acacia; and lubricating agents, for example
25 magnesium stearate, stearic acid or talc. The tablets may be uncoated or they may be coated by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate may be employed.

- 30 Regardless of the route of administration selected, the presently disclosed pharmaceutical compositions may be formulated into pharmaceutically acceptable dosage forms such as described below or by other conventional methods known to those of skill in the art.

In some embodiments, the pharmaceutical composition further comprises an additional active agent. In other embodiments, the additional active agent is selected from the group consisting of an antioxidant, an iron chelator, a statin, an endothelin-1 receptor antagonist, and an angiotensin-converting enzyme inhibitor.

- 5 “Antioxidants” as used herein are compounds which halt or slow chemical oxidation, such as that caused by free radicals, by chemical reduction of reactive free radicals. Antioxidants are often organized into groups, depending on their chemical structures. These groups may include carotenoid terpenoids; flavonoid polyphenols (bioflavonoids); phenolic acids and phenolic acid esters; nonflavonoid phenolics; and
- 10 other organic antioxidants. The carotenoid terpenoids may include, but are not limited to, lycopene, lutein, alpha-carotene, beta-carotene, zeaxanthin and astaxanthin. Flavonoid polyphenols or bioflavonoids may include, but are not limited to, flavanols, such as resveratrol, kaempferol, myricetin, isorhamnetin and proanthocyanadins; the flavones, such as quercetin, rutin, luteolin, apigenin and tangeritin; the flavanones,
- 15 such as hesperetin, naringenin and eriodictyol; the flavan-3-ols, such as catechin, gallocatechin, epicatechin, epigallocatechin, theaflavin and thearubigin; the isoflavone phytoestrogens, such as genistein, diadzein and glycitein; and the anthocyanins, such as cyanidin, delphinidin, malvidin, pelargonidin, peonidin and petunidin. The phenolic acids and phenolic acid esters include, but are not limited to, ellagic acid,
- 20 gallic acid, salicylic acid, rosmarinic acid, chlorogenic acid, chicoric acid, the gallotannins and the ellagitannins. Nonflavonoid phenolic compounds include, but are not limited to, curcumin. Other organic antioxidants may include citric acid, lignan, eugenol, Vitamin A (retinol), Vitamin C (ascorbic acid or calcium ascorbate), Vitamin E (including tocotrienol and tocopherol), alpha-lipoic acid, ascorbyl
- 25 palmitate, butylated hydroxyanisole, butylated hydroxytoluene, hypophosphorous acid, monothioglycerol, propyl gallate, sodium ascorbate, sodium bisulfite, sodium formaldehyde sulfoxylate, sodium metabisulfite, pycnogenol, superoxide dismutase, pine bark grape seed complex, garlic, carotenoids, choline, metabisulfite, catechin, glangin, rutin, luteolin, morin, fisetin, silymerin, ascorbyl palmitate, apigenin,
- 30 ginkgolides, hesperitin, cyanidin, and citrin sodium bisulfite or mixtures thereof.

“Iron chelators” as used herein, are identified by their observed binding to iron and may be classified into bidentate, tridentate, or hexadentate chelators. In particular, dexrazoxane ((S)-(+)-bis-4,4'-(1-methyl-1,2-ethanediyl)2,6-piperazmedione)

is a cardioprotective agent that reduces or prevents myocardial toxicity associated with administration of doxorubicin HCl (Octavia et al. (2012) *J. Mol. Cell Cardiol.* 52(6):1213-25; Octavia et al. (2012) *Free Radic. Biol. Med.* 52(2):291-7). In addition, specific bidentate iron chelators comprise 1,2-dimethyl-3-hydroxypyridin-4-one (Deferiprone, DFP or Ferriprox) and 2-deoxy-2-(N-carbamoylmethyl-[N'-2'-methyl-3'-hydroxypyridin-4'-one])-D-glucopyranose (Feralex-G). Specific tridentate iron chelators comprise pyridoxal isonicotinyl hydrazone (PIH), 4,5-dihydro-2-(2,4-dihydroxyphenyl)-4-methylthiazole-4-carboxylic acid (GT56-252), 4,5-dihydro-2-(3'-hydroxypyridin-2'-yl)-4-methylthiazole-4-carboxylic acid (desferrithiocin or DFT) and 4-[3,5-bis(2-hydroxyphenyl)-[1,2,4]triazol-1-yl]benzoic acid (ICL-670). Substituted 3,5-diphenyl-1,2,4-triazoles in the free acid form, salts thereof and its crystalline forms, as well as formulations of dispersible tablets are disclosed in PCT Patent App. Pub. Nos. WO 97/49395 and WO 2004/035026. Specific hexadentate iron chelators comprise N,N'-bis(o-hydroxybenzyl)ethylenediamine-N,N'-diacetic acid (HBED), N-(5-C3-L (5 aminopentyl) hydroxycarbamoyl)-propionamido)pentyl)-3(5-(N-hydroxyacetoamido)-pentyl)carbamoyl)-propionhydroxamic acid (deferroxamine, desferrioxamine or DFO) and hydroxymethyl-starch-bound deferroxamine (S-DFO). Further derivatives of DFO include aliphatic, aromatic, succinic, and methylsulphonic analogs of DFO and specifically, sulfonamide-deferroxamine, acetamide- deferroxamine, propylamide deferroxamine, butylamide-deferroxamine, benzoylamide- deferroxamine, succinamide-deferroxamine, and methylsulfonamide-deferroxamine.

A further class of iron chelators is the biomimetic class (Meijler et al. (2002) *J. Amer. Chem. Soc.* 124:1266-1267). These molecules are modified analogues of such naturally produced chelators as DFO and ferrichrome. The analogues allow attachment of lipophilic moieties (e.g., acetoxymethyl ester). The lipophilic moieties are then cleaved intracellularly by endogenous esterases, converting the chelators back into hydrophilic molecules which cannot leak out of the cell.

"Statins" as used herein are compounds that inhibit the conversion of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) to mevalonate by inhibiting HMG-CoA reductase, which is an early and rate-limiting step in the cholesterol biosynthetic pathway (Riad et al. (2009) *Cancer Res.* 69(2):695-9). As such, statins are collectively potent lipid lowering agents. Compounds that inhibit the activity of

HMG CoA reductase can be readily identified by using assays well known in the art (e.g., the assays described or cited in U.S. Patent No. 4,231,938 at column 6, and in PCT Patent App. Pub. No. WO 84/02131 at pp. 30-33). Statins include such compounds as atorvastatin, disclosed in U.S. Patent No. 4,681,893, atorvastatin calcium, disclosed in U.S. Patent No. 5,273,995, simvastatin, disclosed in U.S. Patent No. 4,444,784, pravastatin, disclosed in U.S. Patent No. 4,346,227, cerivastatin, disclosed in U.S. Patent No. 5,502,199, mevastatin, disclosed in U.S. Patent No. 3,983,140, velostatin (also called synvinolin), disclosed in U.S. Patent Nos. 4,448,784 and 4,450,171, fluvastatin, disclosed in U.S. Patent No. 4,739,073, compactin, disclosed in U.S. Patent No. 4,804,770, lovastatin, disclosed in U.S. Patent No. 4,231,938, dalvastatin, disclosed in European Patent App. Pub. No. 738510 A2, fluindostatin, disclosed in European Patent App. Pub. No. 363934 A1; dihydrocompactin, disclosed in U.S. Patent No. 4,450,171, rosuvastatin, disclosed in U.S. Patent No. 6,316,460, fluindostatin (Sandoz XU-62-320), and pitavastatin, disclosed in U.S. Patent No. 6,465,477.

“Endothelin receptor antagonists” as used herein refer to compounds that inhibit or block the binding of endothelin with endothelin receptors. Endothelin (ET) is a highly potent vasoconstrictor peptide synthesized and released by the vascular endothelium. Endothelin exists as three isoforms, ET-1, ET-2 and ET-3, of which only ET-1 and ET-3 have been found to be expressed in mammalian systems (unless otherwise stated, “endothelin” as used herein shall mean any or all of the isoforms of endothelin). There are at least two major known endothelin receptors, ET_A and ET_B, both of which are G protein-coupled receptors that when activated result in elevation of intracellular-free calcium (Davenport (2002) *Pharmacol. Rev.* 54(2): 219–26).

Selective ET_A receptor antagonists include sitaxentan (CAS No. 184036-34-8, and as described in Barst et al. (2004) *American J. Resp. Crit. Care Med.* 169(4):441-7); ambrisentan (CAS No. 177036-94-1, and as described in U.S. Patent Nos. 5,703,017, 5,932,730, and 7,109,205); atrasentan (CAS No. 173937-91-2, and as disclosed in U.S. Patent No. 5,767,144); BQ-123 (CAS No. 136553-81-6), and zibotentan (CAS No. 186497-07-4). Dual antagonists of ET_A and ET_B include bosentan (CAS No. 147536-97-8, and as described in Bien et al. (2007) *Cancer Res.* 67(21):10428-35), macitentan (CAS No. 441798-33-0), and tezosentan (CAS No. 180384-57-0).

“Angiotensin-converting enzyme inhibitors” or “ACE inhibitors” as used herein refer to compounds that inhibit or block the conversion of angiotensin I to angiotensin II (Octavia et al. (2012) *J. Mol. Cell Cardiol.* 52(6):1213-25; Hiona et al. (2011) *J. Thorac. Cardiovasc. Surg.* 142(2):396-403). ACE inhibitors include

5 fosinopril or fosinopril sodium (CAS Nos. 98048-97-6 and 88889-14-9, and as disclosed in U.S. Patent No. 4,337,201), ramipril or ramiprilat (CAS Nos. 87333-19-5 and 87269-97-4), captopril (CAS No. 62571-86-2), trandolapril (CAS No. 87679-37-6), moexipril (CAS No. 103775-10-6), lisinopril (CAS No. 83915-83-7), quinapril and quinapril hydrochloride and quinapril diketopiperazine (CAS Nos. 85441-61-8

10 and 82586-55-8 and 103733-49-9), enalapril and enalapril maleate (CAS Nos. 75847-73-3 and 76095-16-4), lisinopril (CAS No. 76547-98-3), perindopril (CAS No. 82834-16-0), and benazepril and benazepril hydrochloride (CAS No. 86541-75-5 and 86541-74-4).

Actual dosage levels of the active ingredients in the presently disclosed

15 pharmaceutical compositions can be varied so as to obtain an amount of the active ingredient that is effective to achieve the desired therapeutic response for a particular subject, composition, route of administration, and disease, disorder, or condition without being toxic to the subject. The selected dosage level will depend on a variety of factors including the activity of the particular compound employed, or

20 pharmaceutically acceptable salt thereof, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compound employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors

25 well known in the medical arts.

Dosages of the compounds used in the presently disclosed subject matter must ultimately be set by an attending physician. General outlines of the dosages are provided herein below. Generally, a suitable dose of an active ingredient in the presently disclosed pharmaceutical compositions, or a pharmaceutically acceptable

30 salt thereof, for administration to a human will be in the range of about 0.1 mg/kg per day to about 500 mg/kg per day (e.g., about .2 mg/kg per day, about .3 mg/kg per day, about .4 mg/kg per day, about .5 mg/kg per day, about .6 mg/kg per day, about .7 mg/kg per day, about .8 mg/kg per day, about .9 mg/kg per day, about 1 mg/kg per

day, about 2 mg/kg per day, about 3 mg/kg per day, about 4 mg/kg per day, about 5 mg/kg per day, about 6 mg/kg per day, about 7 mg/kg per day, about 8 mg/kg per day, about 9 mg/kg per day, about 10 mg/kg per day, about 15 mg/kg per day, about 20 mg/kg per day, about 25 mg/kg per day, about 30 mg/kg per day, about 35 mg/kg per day, about 40 mg/kg per day, about 45 mg/kg per day, about 50 mg/kg per day, about 55 mg/kg per day, about 60 mg/kg per day, about 65 mg/kg per day, about 70 mg/kg per day, about 75 mg/kg per day, about 80 mg/kg per day, about 85 mg/kg per day, about 90 mg/kg per day, about 95 mg/kg per day, about 100 mg/kg per day, about 125 mg/kg per day, about 150 mg/kg per day, about 175 mg/kg per day, about 200 mg/kg per day, about 225 mg/kg per day, about 250 mg/kg per day, about 275 mg/kg per day, about 300 mg/kg per day, about 325 mg/kg per day, about 350 mg/kg per day, about 375 mg/kg per day, about 400 mg/kg per day, about 425 mg/kg per day, about 450 mg/kg per day, or about 475 mg/kg per day). Alternatively, an active ingredient in the presently disclosed pharmaceutical compositions, or a pharmaceutically acceptable salt thereof, for administration to a human will be in the range of from about 1 mg/day to about 500 mg/day; from about 1 mg/day to about 400 mg/day; or from about 1 mg/day to about 300 mg/day. In other embodiments, a suitable dose of an active ingredient in the presently disclosed pharmaceutical compositions, or a pharmaceutically acceptable salt thereof, for administration to a human will be about 1 mg/day, about 2 mg/day, about 3 mg/day, about 4 mg/day, about 5 mg/day, about 6 mg/day, about 7 mg/day, about 8 mg/day, about 9 mg/day, about 10 mg/day, about 15 mg/day, about 20 mg/day, about 25 mg/day, about 30 mg/day, about 35 mg/day, about 40 mg/day, about 45 mg/day, about 50 mg/day, about 55 mg/day, about 60 mg/day, about 65 mg/day, about 70 mg/day, about 75 mg/day, about 80 mg/day, about 85 mg/day, about 90 mg/day, about 95 mg/day, about 100 mg/day, about 125 mg/day, about 150 mg/day, about 175 mg/day, about 200 mg/day, about 225 mg/day, about 250 mg/day, about 275 mg/day, about 300 mg/day, about 325 mg/day, about 350 mg/day, about 375 mg/day, about 400 mg/day, about 425 mg/day, about 450 mg/day, about 475 mg/day, or about 500 mg per day. Dosages may be administered more than one time per day (e.g., two, three, four, or more times per day). If desired, the effective daily dose of the active compound can be administered as two, three, four, five, six or more sub-doses administered separately at appropriate intervals throughout the day, optionally, in unit dosage forms.

III. KITS

In some embodiments, the presently disclosed subject matter provides a kit for treating anthracycline-induced cardiomyopathy, particularly doxorubicin-induced cardiomyopathy, in a subject in need thereof, the kit comprising an NK-1 receptor antagonist and instructions for administration of the NK-1 receptor antagonist to the subject in an amount effective to treat anthracycline-induced cardiomyopathy, particularly doxorubicin-induced cardiomyopathy. In other embodiments, the NK-1 receptor antagonist is aprepitant.

10 In some embodiments, the kit further comprises an anthracycline, particularly doxorubicin, wherein the instructions further comprise instructions for administration of the doxorubicin and the NK-1 receptor antagonist to the subject. In other embodiments, the kit comprises separated dosage units, wherein at least one dosage unit comprises the doxorubicin and at least one other dosage unit comprises the NK-1
15 receptor antagonist. In some other embodiments, the instructions further comprise instructions for simultaneous administration of the doxorubicin and the NK-1 receptor antagonist to the subject. In further embodiments, the instructions further comprise instructions for sequential administration of the doxorubicin and the NK-1 receptor antagonist to the subject. The term “dosage unit” generally refers to the amount of a
20 presently disclosed composition that would be administered to the patient as a single dose.

Typically, the kits of the presently disclosed subject matter comprise a receptor antagonist and instructions for how to perform at least one presently disclosed method. The receptor antagonist is generally supplied in the kits in an
25 amount sufficient to treat at least one patient at least one time to treat anthracycline-induced cardiomyopathy, particularly doxorubicin-induced cardiomyopathy. Alternatively, the kit can be comprised of a dose of receptor antagonist wherein the dose is not enough to treat anthracycline-induced cardiomyopathy, particularly doxorubicin-induced cardiomyopathy, by itself, but when administered with another
30 dose at a later time, it becomes effective. The receptor antagonist supplied in the kits may be supplied as a solution, gel, tablet, capsule, powder, injection, suppository, infusible, lozenge, cream, salve, inhalant, transdermal patch, and the like. The kits

can also comprise some or all of the other reagents and supplies necessary to perform at least one embodiment of the presently disclosed method.

In its simplest form, a kit according to the presently disclosed subject matter comprises a container containing at least one type of receptor antagonist or at least one composition according to the presently disclosed subject matter. Thus, in 5 embodiments, the kit comprises a container containing at least one type of receptor antagonist or a composition comprising a receptor antagonist. In other embodiments, the kit comprises multiple containers, each of which may contain at least one receptor antagonist, compositions comprising receptor antagonists, or other substances that are 10 useful for performing one or more embodiments of the presently disclosed methods.

The container can be any material suitable for containing a presently disclosed composition or another substance useful in performing a presently disclosed method. Thus, the container may be a vial or ampule. It can be fabricated from any suitable material, such as glass, plastic, metal, or paper or a paper product. In embodiments, it 15 is a glass or plastic ampule or vial that can be sealed, such as by a stopper, a stopper and crimp seal, or a plastic or metal cap. In embodiments, the container comprises an effective amount of receptor antagonist to treat anthracycline-induced cardiomyopathy, particularly doxorubicin-induced cardiomyopathy, according to the presently disclosed methods. The amount of receptor antagonist contained in the 20 container can be selected by one of skill in the art without undue experimentation based on numerous parameters that are relevant according to the presently disclosed subject matter.

In embodiments, the container is provided as a component of a larger unit that typically comprises packaging materials (referred to below as a kit for simplicity 25 purposes). The presently disclosed kit can include suitable packaging and instructions and/or other information relating to the use of the compositions. Typically, the kit is fabricated from a sturdy material, such as cardboard and plastic, and can contain the instructions or other information printed directly on it. The kit can comprise multiple containers containing the composition of the invention. In such kits, each container 30 can be the same size, and contain the same amount of composition, as each other container, or different containers may be different sizes and/or contain different amounts of compositions or compositions having different constituents. One of skill in the art will immediately appreciate that numerous different configurations of

container sizes and contents are envisioned by this invention, and thus not all permutations need be specifically recited herein.

In general, the kit comprises containers to contain the components of the kit, and is considered a single package comprising a combination of containers. Thus, the components are said to be in packaged combination within the kit. In addition to a container containing the composition of the invention, the kit can comprise additional containers containing additional compositions of the invention. Each container may contain enough receptor antagonist for a single dose of an embodiment of the method of the invention, or it may contain enough for two or more doses. The various containers may contain differing amounts of the presently disclosed compositions. Thus, in embodiments, the kit comprises a sufficient amount of receptor antagonist to perform an embodiment of the presently disclosed method. The kit can further comprise some or all of the supplies and materials needed to prepare for and perform a presently disclosed method, such as, but not limited to, syringes, sterile water or a sterile aqueous solution. In some embodiments, the kits comprise one or more liquids to hydrate the compositions of the kits.

EXAMPLES

The following Examples have been included to provide guidance to one of ordinary skill in the art for practicing representative embodiments of the presently disclosed subject matter. In light of the present disclosure and the general level of skill in the art, those of skill can appreciate that the following Examples are intended to be exemplary only and that numerous changes, modifications, and alterations can be employed without departing from the scope of the presently disclosed subject matter. The synthetic descriptions and specific examples that follow are only intended for the purposes of illustration, and are not to be construed as limiting in any manner to make compounds of the disclosure by other methods.

EXAMPLE 1

The sensory nerve neuropeptides, substance P and neurokinin A (NKA), are both encoded by alternative splicing of the TAC1 gene (Pennefather et al. (2004) *Life Sciences* 74:1445-1463; Page (2004) *Cell Mol. Life Sci.* 61:1652-1663) and have long been known to have negative inotropic and chronotropic effects on the heart (Hoover

et al. (2000) *Jpn. J. Pharmacol.* 84:367-373; Hoover and Hancock (1988) *J. Auton. Nerv. Syst.* 23:189-197). Recently, there has been renewed interest in the role of sensory nerve neuropeptides in the heart, with D'Souza et al. (D'Souza et al. (2007) *J. Parasitol.* 93:1121-1127) demonstrating that substance P is associated with dilated

5 cardiomyopathy in a mouse model of parasitic myocarditis. They found increased myocardial levels of substance P in the hearts of infected wild type (WT) mice, and hearts from mice deficient in substance P were protected from hypertrophy, cell death, and inflammatory cell infiltration. Robinson et al. (Robinson et al. (2009) *Int. J. Clin. Exp. Med.* 2:76-86) subsequently reported similar findings in a mouse model of viral-

10 induced myocarditis.

Mast cells play a prominent role in initiating adverse myocardial remodelling (Brower et al. (2002) *Am. J. Physiol. Heart Circ. Physiol.* 283:H518-H525; Brower and Janicki (2005) *J. Cardiac Fail.* 11:548-556; Levick et al. (2008) *J. Mol. Cell. Cardiol* 45:56-61; Wei et al. (2003) *Am. J. Physiol. Heart Circ. Physiol.* 285:H784-

15 H792), however, the underlying stimulus responsible for their activation is poorly understood. Until now, no one has investigated the possibility that interactions between substance P and/or NKA and cardiac mast cells mediate myocardial remodelling. A significant number of mast cells in the heart lie in close proximity to nerves (Silver et al. (2004) *PNAS* 101:13607-13612; Arizono et al. (1990) *Lab Invest.* 62:626-634), thus, we hypothesized that sensory nerve neuropeptides may be

20 important activators of mast cells, thereby initiating adverse myocardial remodelling. In this study, we used isolated cardiac mast cells and a model of volume overload induced myocardial remodelling and found that: (i) substance P, but not NKA, induces cardiac mast cell activation via the neurokinin (NK)-1 receptor; (ii) both NK-

25 1 receptor antagonism and deletion of the TAC1 gene prevented adverse remodelling of the left ventricle (LV); and (iii) increased myocardial tumour necrosis factor (TNF)- α and matrix metalloproteinase (MMP) activation were prevented by NK-1 receptor blockade and deletion of TAC1, respectively.

Methods

30 Animals: All of the animal studies conformed to the principles of the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and all protocols were approved by our Institution's Animal Care and Use Committee. Sprague-Dawley rats were obtained from Harlan Laboratories. *TAC1*^{-/-} mice

congenic in the C57BL/6J background were acquired from Jackson Laboratories (Bar Harbor, MA, USA) and a breeding colony was established with genotyping performed according to standard procedures. All animals were housed under standard environmental conditions and maintained on commercial rat or mouse chow and tap water *ad libitum*. Rats and mice were anaesthetized with inhaled isoflurane (3% for rats, 2% for mice) for survival surgeries. Rat terminal surgeries were performed after intra-peritoneal (IP) injection of a combination of ketamine (20 mg) and xylazine (10 mg). Mice terminal surgeries were performed after IP injection of avertin (250 mg/kg). Proper analgesia was evaluated by palpebral reflex, toe pinch reflex, and corneal reflex. At the experimental endpoint euthanasia was accomplished by removal of the heart.

Long-term *in vivo* studies: All experiments were performed using 8-week-old male mice of C57BL/6J background. Volume overload was induced by creation of an aortocaval fistula using a previously described procedure for rats (Brower et al. (2002) *Am. J. Physiol. Heart Circ. Physiol.* 283:H518-H525; Brower and Janicki (2005) *J. Cardiac Fail.* 11:548-556; Brower et al. (1996) *Am. J. Physiol. Heart Circ. Physiol.* 271:H2071-H2078; Brower and Janicki (2001) *Am. J. Physiol. Heart Circ. Physiol.* 280:H674-H683) using a 27½-gauge needle inserted into the abdominal aorta and advanced through the medial wall into the vena cava. The mice were divided into four groups: (i) WT sham (n = 8); (ii) WT fistula (n = 7); (iii) TAC1^{-/-} sham (n = 7); and (iv) TAC1^{-/-} fistula (n = 7). Twenty-eight days post-fistula was chosen as the experimental endpoint based on pilot studies in WT mice, which showed extensive remodelling at this time-point. At the experimental endpoint, the fistula was visually confirmed by identification of turbulent blood flow in the vena cava to ensure that it had remained patent, and the mice were euthanized by removal of the heart. The right ventricle (RV) and LV including septum were separated and weighed. The LV was then sectioned into apical and mid-ventricular sections. The apical section was snap-frozen for biochemical analysis and the mid-ventricular section was fixed in Carnoy's fixative for histological analysis. The lungs were removed and their plural surfaces blotted dry, and weighed.

Echocardiography studies: Echocardiography was performed using a Vevo 660 small animal echocardiographic system (Visual Sonics). Mice were anaesthetized by continual inhalation of 1.5% isoflurane. Measurements of LV

posterior wall thickness and internal chamber diameter were made using two-dimensional M-Mode taken at mid-papillary level. LV function was assessed by fractional shortening (FS), calculated as follows:

$$FS = ((LVIDd - LVIDs)/LVIDd) \times 100,$$

- 5 where LVIDd and LVIDs represent left ventricular internal diameter in diastole and systole, respectively.

Short-term *in vivo* studies: Our previous studies in rats have shown that 3 days post-fistula is a key time-point when mast cell activity is at its peak (Brower et al. (2002) *Am. J. Physiol. Heart Circ. Physiol.* 283:H518-H525). The role of the NK-1 receptor in mediating mast cell-mediated effects was examined using the previously described aortocaval fistula model of volume overload (Brower et al. (2002) *Am. J. Physiol. Heart Circ. Physiol.* 283:H518-H525; Brower and Janicki (2005) *J. Cardiac Fail.* 11:548-556; Levick et al. (2008) *J. Mol. Cell. Cardiol.* 45:56-61; Brower et al. (1996) *Am. J. Physiol. Heart Circ. Physiol.* 271:H2071-H2078). All experiments were performed using 8-week-old male Sprague-Dawley rats randomly divided into three groups: (i) sham-operated (n = 14); (ii) fistula (n = 12); and (iii) fistula + the NK-1 receptor antagonist (L 732 138, 5 mg/kg/day, S.Q., n = 11) beginning 1 day prior to surgery. At 3 days post-surgery, the fistula was visually confirmed by identification of turbulent blood flow in the vena cava to ensure that it had remained patent, and the rats were euthanized and the LV and septum were separated from the RV and weighed. The lungs were removed and their plural surface blotted dry and weighed. A transverse section of the LV was then fixed in Carnoy's fixative and the apical section was snap frozen in liquid nitrogen and stored at -80°C for subsequent analysis.

25 Myocardial TNF- α levels: TNF- α levels were determined from myocardial samples using a commercially available ELISA kit (BD Biosciences). Protein was extracted from myocardial tissue by homogenization followed by sonication. Each sample was then incubated with triton-X before being separated into cytosolic/extracellular and membrane fractions by centrifugation. TNF- α was measured in the cytosolic/extracellular fraction with each sample run in duplicate.

30 Mast cell density and collagen volume fraction: Five micrometre thick coronal sections were stained with the mast cell-specific stain, toluidine blue. Mast cell density was determined by dividing the total number of mast cells per LV cross-

section by the tissue area of the corresponding section. Collagen volume fraction was determined as previously described (Meléndez et al. (2010) *Hypertension* 56:225-231; Levick et al. (2009) *Hypertension* 53:1041-1047; Levick et al. (2010) *Hypertension* 55:270-276; Levick et al. (2011) *Cardiovasc. Res.* 89:12-19) with 5 μm thick paraffin-
 5 embedded sections stained with picosirius red (0.1% Sirius Red F3BA in picric acid) following incubation in phosphomolybdic acid (0.2%). Twenty random images per LV section were acquired and analysed with Image J software (NIH). Perivascular areas were excluded from the collagen analysis.

TUNEL assay: Terminal deoxynucleotidyl transferase dUTP nick end
 10 labelling (TUNEL) (Roche Diagnostics) was applied to tissue sections and the amount of apoptotic nuclei per tissue section was quantified. Slides were also co-stained with DAPI (Sigma) to verify nuclei presence. Counts of TUNEL positive cells normalized to LV area were used to quantify changes in cell death between groups.

Cardiac inflammatory cell isolation procedure (*in vitro* studies): Cardiac
 15 inflammatory cells were isolated from rat hearts as previously described (Morgan et al. (2008) *Inflamm. Res.* 57:1-6). Briefly, a thoracotomy exposed the intact pericardial sac, which was then filled with Hanks balanced salt solution (HBSS, 7.4 pH; Sigma Aldrich) using a teflon catheter sleeve attached to a sterile 10 cc syringe. The buffer was then aspirated into a new sterile 10 cc syringe. This was repeated several times.
 20 This procedure results in the collection of predominantly mast cells, T cells, and monocyte/macrophages (Levick et al. (2010) *Hypertension* 55:270-276). 2×10^5 of this mixed population of cells per well were incubated with substance P (100 μM) for 20 h in Dulbeccos Modified Eagle media containing 10% FBS, penicillin, streptomycin, amphotceterin B, and gentamicin. At completion of the incubation
 25 period, the media was assayed for TNF- α using a commercially available TNF- α ELISA kit (BD Biosciences). Specific cardiac mast cell responses were examined by incubating 4×10^3 mast cells per treatment tube in HyClone buffer (Thermo Scientific) containing substance P (0, 3×10^{-6} , 1×10^{-5} , 3×10^{-5} , 1×10^{-4} , and 3×10^{-4} μM) or NKA (0, 3×10^{-6} , 1×10^{-5} , 3×10^{-5} , and 1×10^{-4} μM) at 37°C for 20
 30 min. The post-treatment supernatants and pellets were separated for subsequent analysis of histamine as a marker of mast cell degranulation using a commercial ELISA kit (Neogen, Lexington, KY, USA). Per cent histamine release was determined by dividing the histamine value from the supernatant by total histamine

(supernatant plus pellet). To determine the contribution of NK-1 and -2 receptors to the activation of cardiac mast cells, additional groups of isolated cells were pre-incubated for 20 min with the NK-1 or -2 receptor antagonists, L 732 138 (20 μ M) and GR 159897 (10 μ M), respectively, before treatment with substance P (100 μ M).

5 Statistical analysis: All grouped data were expressed as mean $\pm$ SD or SEM as appropriate. Grouped data comparisons were made by one-way ANOVA, using SPSS 11.5 software (SPSS, Inc., Chicago, IL, USA). When a significant *F*-test ($P < 0.05$) was obtained, intergroup comparisons were analysed using the Fisher protected least-significant difference *post hoc* testing. Statistical significance was taken to be $P <$
10 0.05.

Results

Long-term myocardial remodeling in TAC^{-/-} mice: The results for body, LV, RV, and lung weight are displayed in Table 1. Volume overload in the WT led to a
15 significant increase in all parameters measured when compared with the WT sham animals. This is indicative of a progression towards heart failure in the WT fistula mice. The TAC1^{-/-} mice were slightly smaller than their WT counterparts. In contrast to the WT, none of the parameters in the TAC1^{-/-} mice with fistula was significantly different from those in the TAC1^{-/-} sham mice; moreover, all parameters were
20 significantly different from the WT fistula animals.

Table 1. Biometric parameters.

Groups	n	BW (g)	LV weight (mg)	RV weight (mg)	Lung weight (mg)
WT sham	8	26 $\pm$ 1	90.9 $\pm$ 10.1	23.8 $\pm$ 2.4	138.3 $\pm$ 21.2
WT fistula	7	29 $\pm$ 1*	111.9 $\pm$ 24*	33.6 $\pm$ 8.9*	160.4 $\pm$ 19.9*
TAC1 ^{-/-}	7	24 $\pm$ 1***	78.4 $\pm$ 3.5**	20.4 $\pm$ 1.3**	131.9 $\pm$ 10.7**

Groups	n	BW (g)	LV weight (mg)	RV weight (mg)	Lung weight (mg)
sham					
TAC1 ^{-/-} fistula	7	25 ± 3**	80.9 ± 8.2**	22.9 ± 3.1**	130.1 ± 9.3**

Long-term studies in mice at 28 days post-fistula and their respective shams.

All values are mean ± SD. BW, body weight; LV, left ventricle; RV, right ventricle.

*P < 0.05 vs. WT sham.

**P < 0.05 vs. WT fistula.

- 5 Echocardiography: Heart rate was not significantly different between WT sham and WT fistula mice at any time-point measured (Figure 1A). Heart rates of the TAC1^{-/-} groups did not differ significantly from each other, however, both TAC1^{-/-} sham and fistula mice had significantly lower heart rates than WT sham and WT fistula mice at 14 and 28 days post-fistula. LV chamber diameter and wall thickness
- 10 are expressed both as absolute values and as per cent change from baseline. Following fistula, WT hearts showed a continual increase in LV chamber size reaching 5.3 ± 0.2 mm, which represents a 31.9% increase above baseline at 28 days post-fistula (Figure 1B and C). In contrast, WT sham hearts increased in chamber dimension above baseline by only 6.5% (reaching 4.4 ± 0.1 mm), likely indicative of
- 15 normal growth. TAC1^{-/-} sham hearts as well as TAC1^{-/-} fistula hearts increased by 4.1% (reaching 3.8 ± 0.1 mm) and 6.3% (reaching 4.1 ± 0.1 mm), respectively. Thus, there were no significant differences between these two groups. While there was no significant difference in the size or per cent change for LV posterior wall thickness,
- 20 there was a definite trend towards wall thinning in the WT fistula group (Figure 1D and E). This trend was not apparent in the TAC1^{-/-} fistula group. FS was determined as a measure of cardiac function (Figure 1F). While there was no statistically significant difference between WT sham and WT fistula groups at 28 days post-fistula, there was a definite trend towards a decrease in FS in the WT fistula group. This trend was not present in the TAC1^{-/-} fistula group.
- 25 Myocardial TNF- α levels: There were no significant differences in TNF- α in the WT groups (Figure 2A). Myocardial levels of TNF- α in the TAC1^{-/-} fistula were

significantly lower than the WT fistula. Overall, TAC1^{-/-} mice had lower levels of TNF- α .

Myocardial MMP activity: MMP activity (arbitrary units) was significantly increased in the WT fistula group when compared with WT sham group (Figure 2B).

5 The TAC1^{-/-} fistula group was not significantly different from the TAC1^{-/-} sham.

TUNEL assay: TUNEL staining was used to identify dying cells in the myocardium. There were no significant differences between both fistula groups and their respective controls (Figure 2C). Both the TAC1^{-/-} sham and fistula groups had more TUNEL⁺ cells/mm² than the WT fistula group.

10 Collagen volume fraction: Collagen volume fraction was significantly decreased at 28 days post-fistula in WT fistula mice when compared with the WT sham (Figure 3A and B). This decrease in collagen volume fraction did not occur in the TAC1^{-/-} fistula animals compared with the TAC1^{-/-} shams.

Degranulation of cardiac mast cells: The ability of substance P and NKA to
15 induce cardiac mast cell degranulation was examined using isolated cardiac mast cells. The concentration–response curves for histamine release are displayed in Figure 4A. Substance P elicited a strong concentration-dependent secretagogue effect with the maximum per cent of histamine released being $64 \pm 4\%$ (EC₅₀ –log 4.4). In contrast, cardiac mast cells released essentially no histamine in response to NKA.
20 Pre-treatment with the selective NK-1 receptor antagonist, L 732 138 prior to stimulation with substance P prevented the release of histamine (Figure 4B). Pre-treatment with the selective NK-2 receptor antagonist, GR 159 897 had no effect. Neither L 732 138 or GR 159 897 had any effect on histamine release when administered alone (data not shown).

25 Substance P-induced release of TNF- α from isolated cardiac inflammatory cells: A mixed population of isolated cardiac inflammatory cells containing T cells, mast cells, and macrophages was stimulated with substance P (100 μ M) to investigate the ability of substance P to induce TNF- α release. Substance P was found to significantly increase TNF- α production (Figure 4C).

30 Effect of NK-1 receptor antagonism on short-term myocardial remodelling, cardiac mast cell density and myocardial TNF- α in rats: The importance of the NK-1 receptor in the initial phase of volume overload-induced adverse myocardial remodelling, when mast cell density is at its greatest, was investigated using the

fistula model. At 3 days post-fistula there were no changes in body, RV or lung weight (Table 2). However, LV weight was increased in the untreated and treated fistula groups. No biometric parameters were affected by treatment with L 732 138 in any group. In untreated fistula rats at 3 days post-fistula, there was a characteristic decrease in collagen volume fraction when compared with sham-operated controls, indicative of collagen degradation (Figure 5A and B). This degradation of collagen was prevented by the selective NK-1 receptor antagonist, L 732 138. Cardiac mast cell density was increased in untreated fistula rats compared with shams (Figure 5C). Treatment with L 732 138 prevented this increase in mast cell density. Creation of a fistula caused a significant increase in myocardial TNF- α levels at 3 days post-fistula (Figure 5D). NK-1 receptor blockade prevented the increased levels of TNF- α in the fistula.

Table 2: Biometric parameters.

Groups	n	BW (g)	LV weight (mg)	RV weight (mg)	Lung weight (mg)
Sham	14	262 $\pm$ 13	595.9 $\pm$ 38.4	163.9 $\pm$ 11.0	1421.8 $\pm$ 198.3
Fistula	12	289 $\pm$ 41	687.2 $\pm$ 81.0*	182.2 $\pm$ 38.4	1446.1 $\pm$ 164.9
Fistula + L732138	11	269 $\pm$ 17	665.6 $\pm$ 78.8*	185.1 $\pm$ 26.8	1322.0 $\pm$ 78.2

Short-term studies were done in rats at 3 days post-fistula and their respective shams. All values are mean $\pm$ SD. BW, body weight; LV, left ventricle; RV, right ventricle. P < 0.05 vs sham.

Discussion: In addition to the central nervous system, substance P and NKA are often co-localized in peripheral sensory nerves (Pennefather et al. (2004) *Life Sciences* 74:1445-1463) with these nerves being associated with numerous areas of the heart, including the ventricle, atria, valves, and connective linings (Furness et al. (1984) *Clin. Exp. Hypertens. A* 6:91-106. In this study, we used the fistula model of volume overload to assess the effect of deletion of the TAC1 gene, which encodes for substance P and NKA, on myocardial remodelling. Although a fistula is not a common clinical condition, the pattern of remodelling that occurs in this model mimics that of the human myocardium in response to volume overload (Grossman et

al. (1975) *J. Clin. Invest.* 56:56-64). That is, a hypertrophic remodelling that is insufficient to normalize diastolic wall stress and ultimately can no longer meet the demands of the body for blood supply, and as a result edematous increases in lung and body weights become apparent (Brower and Janicki (2005) *J. Cardiac Fail.* 11:548-556; Levick et al. (2008) *J. Mol. Cell Cardiol.* 45:56-61; Brower et al. (1996) *Am. J. Physiol. Heart Circ. Physiol.* 271:H2071-H2078; Huang et al. (1992) *Am. J. Physiol. Heart Circ. Physiol.* 262:H846-H851). The first finding of the current study is that in contrast to WT animals, TAC1^{-/-} mice did not develop adverse structural remodelling following induction of volume overload. Echocardiographic analysis revealed that TAC1^{-/-} hearts did not progressively dilate as is characteristic of volume overload. Further, WT fistula hearts showed a decreasing FS over the final 14 days of the 28 day study period. Although this decrease did not reach significance, this together with the increase in body, LV, RV, and lung weights, strongly suggests that these hearts were beginning to fail. This trend towards a decreased FS was absent in the TAC1^{-/-} mice and they did not have significant increases in body, LV, RV, or lung weights. D'Souza et al. (D'Souza et al. (2007) *J. Parasitol.* 93:1121-1127) had previously used mice that did not express substance P to investigate remodelling of the heart in response to *Taenia crassiceps* infection. While they did not identify the specific gene deleted in these mice, it was more than likely TAC1 since this is the only gene that encodes substance P. They found that unlike the WT, their knockout mice did not develop ventricular hypertrophy following infection. Similarly, Robinson et al. (Robinson et al. (2009) *Int. J. Clin. Exp. Med.* 2:76-86) also observed that myocardial hypertrophy was prevented following infection with encephalomyocarditis virus in mice not expressing substance P. In these myocarditis studies, the authors attributed a decrease in cell death as a possible mechanism of protection in the knockout animals; however, we found cell death not to be important in our model. This may reflect differences between myocarditis-induced remodelling and remodelling induced by increased volume overload. Instead, we found that deletion of the TAC1 gene prevented the increased activity of MMPs that was present in the WT fistula. Many MMPs are active during the remodelling process and MMPs are critical to initiating collagen degradation and inducing subsequent LV dilatation (Spinale (2007) *Physiol. Rev.* 87:1285-1342). In keeping with this lack of MMP activation, hearts from TAC1^{-/-} fistula animals did not undergo collagen degradation.

This prevention of MMP activity by deletion of TAC1 is consistent with observations in human lung fibroblasts where substance P has been shown to increase MMP-1 (collagenase) and collagen degradation (Ramos et al. (2007) *Exp. Lung Res.* 33:151-167), and human gingival fibroblasts where substance P increased the quantity of numerous MMPs (Cury et al. (2008) *J. Periodontal Res.* 43:255-260). Volume overload also induces myocardial TNF- α production (Jobe et al. (2009) *Am. J. Physiol. Heart Circ. Physiol.* 297:H1462-H1468), which is critical in driving adverse myocardial remodelling, including the degradation of collagen (Jobe et al. (2009) *Am. J. Physiol. Heart Circ. Physiol.* 297:H1462-H1468; Bozkurt et al. (1998) *Circulation* 97:1382-1391). However, TNF- α was not significantly elevated at 28 days post-fistula in either group. However, this does not rule out the possibility that TNF- α was modulated by TAC1 at an earlier time-point as was the case with the 3 day fistula animals.

Cardiac mast cells are known to be important in driving adverse myocardial remodelling (Brower et al. (2002) *Am. J. Physiol. Heart Circ. Physiol.* 283:H518-H525; Brower and Janicki (2005) *J. Cardiac Fail.* 11:548-556; Levick et al. (2008) *J. Mol. Cell Cardiol.* 45:56-61; Wei et al. (2003) *Am. J. Physiol. Heart Circ. Physiol.* 285:H784-H792). Mast cells are often spatially located close to nerves (Silver et al. (2004) *PNAS* 101:13607-13612; Arizono et al. (1990) *Lab Invest.* 62:626-634) and a wide range of mast cells are known to respond to substance P (Morgan et al. (2008) *Inflamm Res.* 57:1-6; Guhl et al. (2005) *J. Neuroimmunol.* 163:92-101; Shanahan et al. (1985) *J. Immunol.* 135:1331-1337; Ottosson and Edvinsson (1997) *Cephalalgia* 17:166-174; Heaney et al. (1995) *Clin. Exp. Allergy* 25:179-186). With this in mind, we sought to determine whether substance P and NKA could activate isolated cardiac mast cells. Herein, we demonstrate that substance P elicited a strong concentration-dependent secretagogue effect on isolated cardiac mast cells, mediated via the NK-1 receptor. Conversely, NKA elicited virtually no response. NKA binds to NK-2 receptors, and the very small histamine release observed with NKA, coupled with the finding that the NK-2 receptor antagonist did not prevent cardiac mast cell degranulation in response to substance P, suggests that NK-2 receptors may not be present on cardiac mast cells. In fact, a study of rat hearts has previously revealed a lack of expression of the NK-2 receptor in this organ (Candenas et al. (2002) *Life Sci.* 72:269-277). Having identified substance P and not NKA as the more likely mediator

of mast cell activation and hence myocardial remodelling, we wanted to test the effects of substance P on TNF- α release. Since we also know that all inflammatory cells in the heart produce TNF- α following volume overload (Murray et al. (2010) *J Mol Cell Cardiol* 49:245-250), and substance P is known to stimulate production of TNF- α by numerous cell types including mast cells and lymphocytes (Azzolina et al. (2003) *Mol. Cell Res.* 1643:75-83; Ansel et al. (1993) *J. Immunol.* 150:4478-4485; Joachim et al. (2006) *Neuroimmunomodulation* 13:43-50), we stimulated with substance P a mixed population of inflammatory cells (lymphocytes, mast cells, and macrophages) (Levick et al. (2010) *Hypertension* 55:270-276) isolated from rat hearts. As expected, TNF- α release was increased following stimulation. While we cannot rule out non-specific effects due to the high concentration of substance P required to induce an effect, TNF- α release was not due to changes in cell viability.

Having determined that substance P activation of cardiac mast cells occurred via the NK-1 receptor, we sought to determine the importance of this receptor to myocardial remodelling in vivo. Peak mast cell activity occurs at ~3 days post-fistula, with concomitant collagen degradation (Brower et al. (2002) *Am. J. Physiol. Heart Circ. Physiol.* 283:H518-H525). It is this early mast cell activation that initiates the long-term remodelling of the heart (Brower and Janicki (2005) *J. Cardiac Fail.* 11:548-556). Accordingly, we treated rats with the NK-1 receptor antagonist, L 732 138 for 3 days post-fistula and found that blockade of this receptor prevented mast cell density from increasing. Consequently, collagen degradation did not occur. NK-1 receptor blockade also prevented fistula-induced increases in myocardial TNF- α , consistent with our *in vitro* findings that substance P induces TNF- α production.

In summary, we demonstrate for the first time that sensory nerve neuropeptides mediate adverse myocardial remodelling via a mechanism involving cardiac mast cells, TNF- α , and MMPs (Figure 6). While we cannot say definitively that it is substance P and not NKA that is the critical neuropeptide, evidence presented in this study would suggest that this is the case. Clearly these results need to be tested in other models of cardiac disease, such as pressure overload, since there are clear differences in the remodelling processes. While both volume and pressure overload induce a similar degree of hypertrophy in response to similarly elevated wall stresses, both differ in patterns of gene regulation, calcium handling, and extracellular matrix response (Toischer et al. (2010) *Circulation* 122:993-1003), indicating that different

loads may require specific pharmacological interventions. However, if these findings should subsequently be found to be relevant to human cardiac disease, then these findings are particularly exciting and relevant given that antagonists of the NK-1 receptor are already in use at the clinical trial level, undergoing testing for the prevention of post-operative nausea and vomiting (Gan et al. (2007) *Anesth. Analg.* 104:1082-1089, depression (Kramer et al. (2004) *Neuropsychopharmacology* 29:385-392; Keller et al. (2006) *Biolog. Psychiat.* 59:216-223), diabetic neuropathy (Sindrup et al. (2006) *Euro. J. Pain* 10:567-571), chemotherapy-induced nausea (de Wit et al. (2004) *Euro. J. Cancer* 40:403-410; Hesketh et al. (2003) *J. Clin. Oncol.* 21:4112-4119), and migraines (Goldstein et al. (2001) *Cephalalgia* 21:102-106), and thus may represent a treatment strategy for the prevention of adverse cardiac remodelling in the foreseeable future.

EXAMPLE 2

Determine whether the neuropeptide substance P (SP) mediates adverse myocardial remodeling induced by anthracyclines (DOX) and whether blockage of the neurokinin-1 (NK-1) receptor (the receptor for SP) prevents and/or reverse adverse outcomes (i.e. myocardial fibrosis). To accomplish this, a combination of *in vitro* and *in vivo* experiments will be designed (Melendez et al. (2011) *Cardiovasc. Res.* 92(3):420-9). Mouse and/or rat models of anthracycline induced cardiomyopathy will be utilized to confirm myocardial SP upregulation as well as activation of pro-fibrotic molecular pathways of myocardial remodeling induced by anthracyclines *in vivo* (Lightfoot et al. (2010) *Circ. Cardiovasc. Imaging* 3(5):550-8; Singla et al. (2012) *Cell Transplant.* 21(9):1919-30). Pretreatment of animals with the NK-1 receptor antagonist (aprepitant) would result in prevention or attenuation of myocardial remodeling (myocardial fibrosis) (Melendez et al. (2011) *Cardiovasc. Res.* 92(3):420-9).

The *in vitro* studies will consist in two innovative types of culture systems: the first will use isolated cardiac fibroblasts from rats or mice in a 3-dimensional magnetic levitation culture system which recreates the natural environment of the cells and it is more physiologically relevant to examine the effects of DOX and/or SP on extracellular matrix (ECM) gene expression. The supernatants of the cultures will be examined to determine levels of hydroxyproline as well as other products of ECM

synthesis by fibroblasts. The NK-1 receptor antagonists as well as other potential modulators will be tested in this experimental setting (Haisler (2013) *Nat. Protoc.* 8(10):1940-9). The second culture system will consist in a 3-dimensional left ventricular slice culture where 200-250 μ m slices of left ventricle (LV) are maintained in culture for up to 48 hours. Using this technique, the structural environment of the myocardium will be preserved and will allow fibroblasts to interact with other myocardial cells while preserving a highly controlled experimental system. The supernatants of these cultures will be evaluated for ECM components and inflammatory cytokines. The LV slices will be evaluated using histomorphometric and immunohistochemistry approaches to determine the effects of DOX and SP on LV inflammatory cells, myocytes and fibroblasts. Using these culture approaches the cellular and molecular pathophysiologic pathways will be dissected and will determine the *in vivo* experimental approach.

The *in vivo* studies will utilize rat and/or mouse models of doxorubicin cardiomyopathy to test the findings from the previously described *in vitro* studies. Rats and/or mice will be pretreated with the NK-1 receptor antagonist. At the end of the experimental period, cardiac function will be evaluated with cardiac magnetic resonance (CMR). Similarly, an ex vivo assessment of LV size and function using a blood-perfuse isolated heart preparation (modified Langerdorff heart preparation) (Brower (2005) *J. Card. Fail.* 11(7):548-56; Melendez et al. (2010) *Hypertension* 56(2):225-31). A slice of LV tissue will be snap-frozen in liquid nitrogen for further biochemical analysis and the remaining tissue will be processed for routine histopathology and collagen volume fraction (CVF). Serum samples will be collected for assessment of biomarkers (Melendez et al. (2011) *Cardiovasc. Res.* 92(3):420-9; Melendez et al. (2010) *Hypertension* 56(2):225-31).

EXAMPLE 3

The effect of one or more additional active agents when administered with aprepitant in preventing or attenuating anthracycline induced myocardial remodeling will be determined. SP is an upstream regulator of remodeling (Melendez et al. (2011) *Cardiovasc. Res.* 92(3):420-9; Dehlin et al. (2013) *Int. J. Cardiol.* 168(5):4643-51; Dehlin et al. (2014) *Int. J. Cardiol.* 170(3):270-7). It is possible that alternative molecular remodeling pathways and mechanisms of myocardial injury

may also become activated after treatment with anthracyclines (El-Boghdady (2013) *J. Biochem. Biophys.* 50(3):202-9; Octavia et al. (2012) *J. Mol. Cell. Cardiol.* 52(6):1213-25).

Additional active agents include: antioxidants, iron chelators (dexrazoxane) (Octavia et al. (2012) *J. Mol. Cell Cardiol.* 52(6):1213-25; Octavia et al. (2012) *Free Radic. Biol. Med.* 52(2):291-7), 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors (statins) (Riad et al. (2009) *Cancer Res.* 69(2):695-9), endothelin-1 receptor antagonist (Bosentan) (Bien et al. (2007) *Cancer Res.* 67(21):10428-35) and angiotensin-converting enzyme inhibitors (ACEIs) (Octavia et al. (2012) *J. Mol. Cell Cardiol.* 52(6):1213-25; Hiona et al. (2011) *J. Thorac. Cardiovasc. Surg.* 142(2):396-403).

Experiments described in Example 2 will be repeated using a combination of aprepitant and the additional active agent.

15 EXAMPLE 4

To assess the translational relevance of the previous findings, non-human primate placebo-control randomized studies will be conducted to determine if pretreatment with aprepitant alone or combined with a additional active agent, attenuates myocardial remodeling in monkeys treated with anthracyclines. A pilot project to determine the optimal dose of aprepitant to prevent adverse myocardial remodeling will be conducted before the initiation of the monkey preclinical trial.

Using a monkey model of DOX induced cardiomyopathy, four groups of monkeys will be randomized to receive 1) DOX + Placebo, 2) DOX + Aprepitant (dose previously determined), 3) DOX + Aprepitant + Additional active agent (as determined in Example 4) or 4) DOX + Aprepitant + Additional active agent alone (as determined in Example 3). We will use innovative noninvasive magnetic resonance imaging (CMR) procedures to accurately measure cardiac function in each group pre and post administration of Dox (Lightfoot et al. (2010) *Circ. Cardiovasc. Imaging* 3(5):550-8; Stacey et al. (2013) *Curr. Treat. Options. Cardiovasc. Med.* 15(4):373-86; Drafts et al. (2013) *JACC. Cardiovasc. Imaging* 6(8):877-85). Additionally LV volume, myocardial strain, fibrosis, aortic pulse wave velocity and LV wall thickness, all factors that can influence LV function will be measured (Banchs (2011) *Tex. Heart Inst. J.* 38(3):268-9). Advanced serum biomarkers will be

determined to assess for systemic inflammation and circulating neurohormones that may be modulated by aprepitant. At the end of experimental design, monkeys will be necropsied. Confirmation of presence of cardiomyopathy will be achieved with biochemical analyses of snap-frozen tissue as well as routine histopathologic and immunohistochemistry evaluations. Pretreatment with aprepitant would result in preservation of LV function and prevention of myocardial fibrosis.

EXAMPLE 5

The results of Example 4 will be used to plan a randomized double-blind, placebo-controlled, clinical trial that will be designed to determine whether pretreatment with aprepitant alone, or in combination with adjuvant drugs prevents or attenuate the reduced cardiac function observed in patients treated with DOX-based chemotherapy for cancer. A team of clinical cardiologists, epidemiologists and cardiovascular investigators will be ensemble to perform this study. Briefly pre and post anthracycline treatment evaluation of LV function using non-invasive magnetic resonance procedures will be performed. Factors that influence LV contractility and ejection fraction include LV wall thickness, LV volumes, pulse wave velocity, myocardial strain and myocardial extracellular volume (ECV) (Banchs (2011) *Tex. Heart Inst. J.* 38(3):268-9). Serum biomarkers will be measure to assess for presence of oxidate stress, systemic inflammation and circulating neurohormones that may influence myocardial remodeling. The results of the rodent and non-human primate studies are essential to determine the endpoints and details of the clinical trial. A systematic approach for human studies will be designed after the completion of Example 5.

REFERENCES

All publications, patent applications, patents, and other references mentioned in the specification are indicative of the level of those skilled in the art to which the presently disclosed subject matter pertains. All publications, patent applications, patents, and other references are herein incorporated by reference to the same extent as if each individual publication, patent application, patent, and other reference was specifically and individually indicated to be incorporated by reference. It will be understood that, although a number of patent applications, patents, and other

references are referred to herein, such reference does not constitute an admission that any of these documents forms part of the common general knowledge in the art.

5 Although the foregoing subject matter has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be understood by those skilled in the art that certain changes and modifications can be practiced within the scope of the appended claims.

THAT WHICH IS CLAIMED:

1. A method for treating anthracycline-induced cardiomyopathy in a subject in need thereof, the method comprising administering to the subject an effective amount of a neurokinin-1 (NK-1) receptor antagonist.
2. The method of claim 1, wherein the anthracycline and the NK-1 receptor antagonist are administered simultaneously to the subject.
3. The method of claim 1, wherein the anthracycline and the NK-1 receptor antagonist are administered sequentially to the subject.
4. The method of claim 3, wherein the NK-1 receptor antagonist is administered prior to the anthracycline administration.
5. The method of claim 3, wherein the NK-1 receptor antagonist is administered after the anthracycline administration.
6. The method of claim 3, wherein the NK-1 receptor antagonist is administered for at least 2 consecutive days after treatment with the anthracycline.
7. The method of any one of claims 1 to 6, wherein the anthracycline is selected from the group consisting of doxorubicin, daunorubicin, epirubicin, idarubicin, and valrubicin, or a pharmaceutically acceptable salt thereof.
8. The method of any one of claims 1 to 7, wherein the NK-1 receptor antagonist is aprepitant, or a pharmaceutically acceptable salt thereof.
9. The method of any one of claims 1 to 8, further comprising administration of an additional active agent.

10. The method of claim 9, wherein the additional active agent is selected from the group consisting of an antioxidant, an iron chelator, a statin, an endothelin-1 receptor antagonist, and an angiotensin-converting enzyme inhibitor.

11. A pharmaceutical composition comprising an anthracycline, an NK-1 receptor antagonist, and a pharmaceutically acceptable carrier.

12. The pharmaceutical composition of claim 11, wherein the anthracycline is selected from the group consisting of doxorubicin, daunorubicin, epirubicin, idarubicin, and valrubicin, or a pharmaceutically acceptable salt thereof.

13. The pharmaceutical composition of any one of claims 11 to 12, wherein the NK-1 receptor antagonist is aprepitant, or a pharmaceutically acceptable salt thereof.

14. The pharmaceutical composition of any one of claims 11 to 13, further comprising an additional active agent.

15. The pharmaceutical composition of claim 14, wherein the additional active agent is selected from the group consisting of an antioxidant, an iron chelator, a statin, an endothelin-1 receptor antagonist, and an angiotensin-converting enzyme inhibitor.

16. A kit for treating anthracycline-induced cardiomyopathy in a subject in need thereof, the kit comprising an NK-1 receptor antagonist and instructions for administration of the NK-1 receptor antagonist to the subject in an amount effective to treat anthracycline-induced cardiomyopathy.

17. The kit of claim 16, wherein the NK-1 receptor antagonist is aprepitant, or a pharmaceutically acceptable salt thereof.

18. The kit of any one of claims 16 to 17, further comprising an anthracycline, wherein the instructions further comprise instructions for administration of the anthracycline and the NK-1 receptor antagonist to the subject.

19. The kit of claim 18, wherein the anthracycline is selected from the group consisting of doxorubicin, daunorubicin, epirubicin, idarubicin, and valrubicin, or a pharmaceutically acceptable salt thereof.

20. The kit of any one of claims 16 to 19, wherein the kit comprises separated dosage units, wherein at least one dosage unit comprises the anthracycline and at least one other dosage unit comprises the NK-1 receptor antagonist.

21. The kit of claim 20, wherein the instructions further comprise instructions for simultaneous administration of the anthracycline and the NK-1 receptor antagonist to the subject.

22. The kit of claim 20, wherein the instructions further comprise instructions for sequential administration of the anthracycline and the NK-1 receptor antagonist to the subject.

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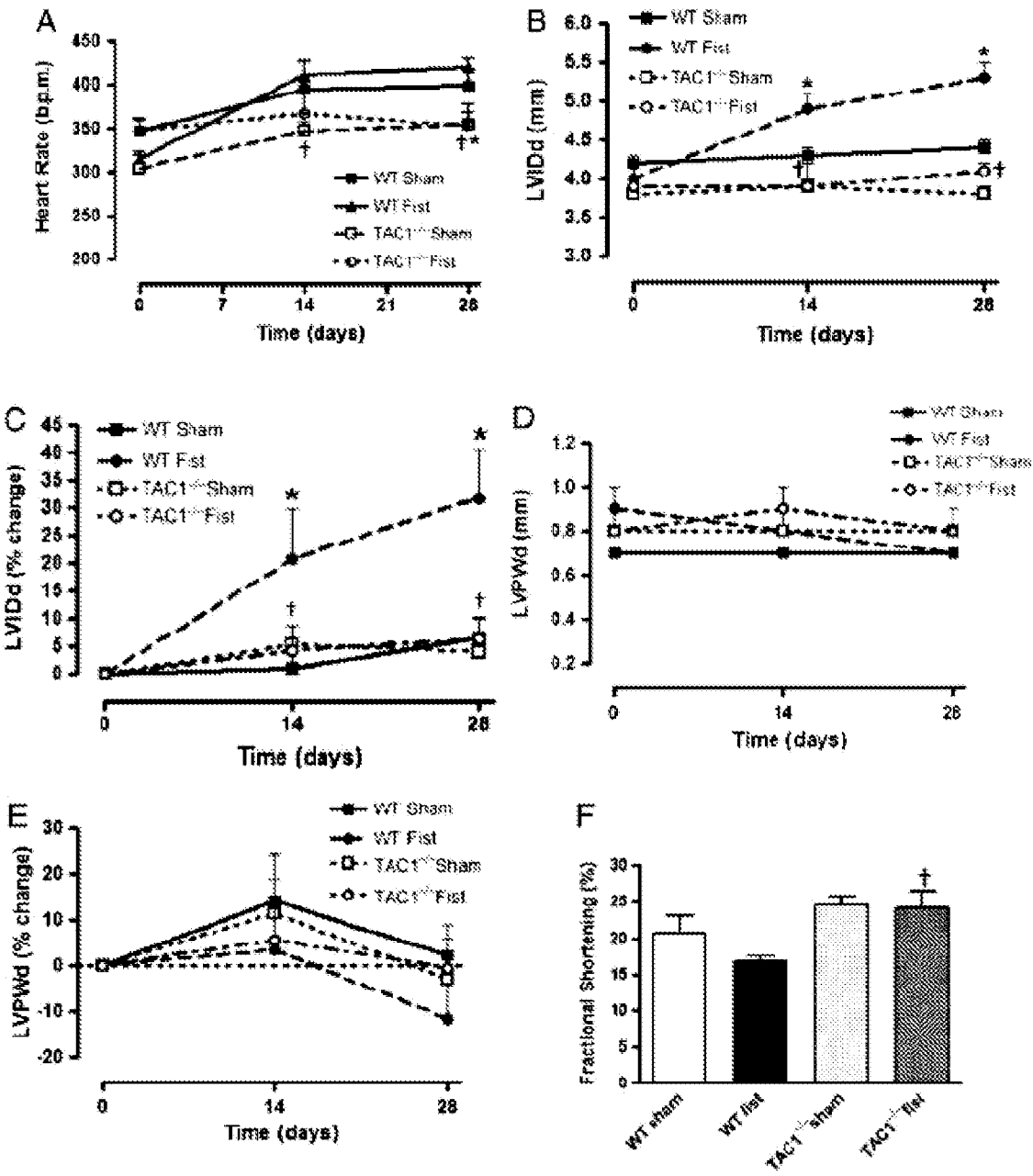


FIG. 1

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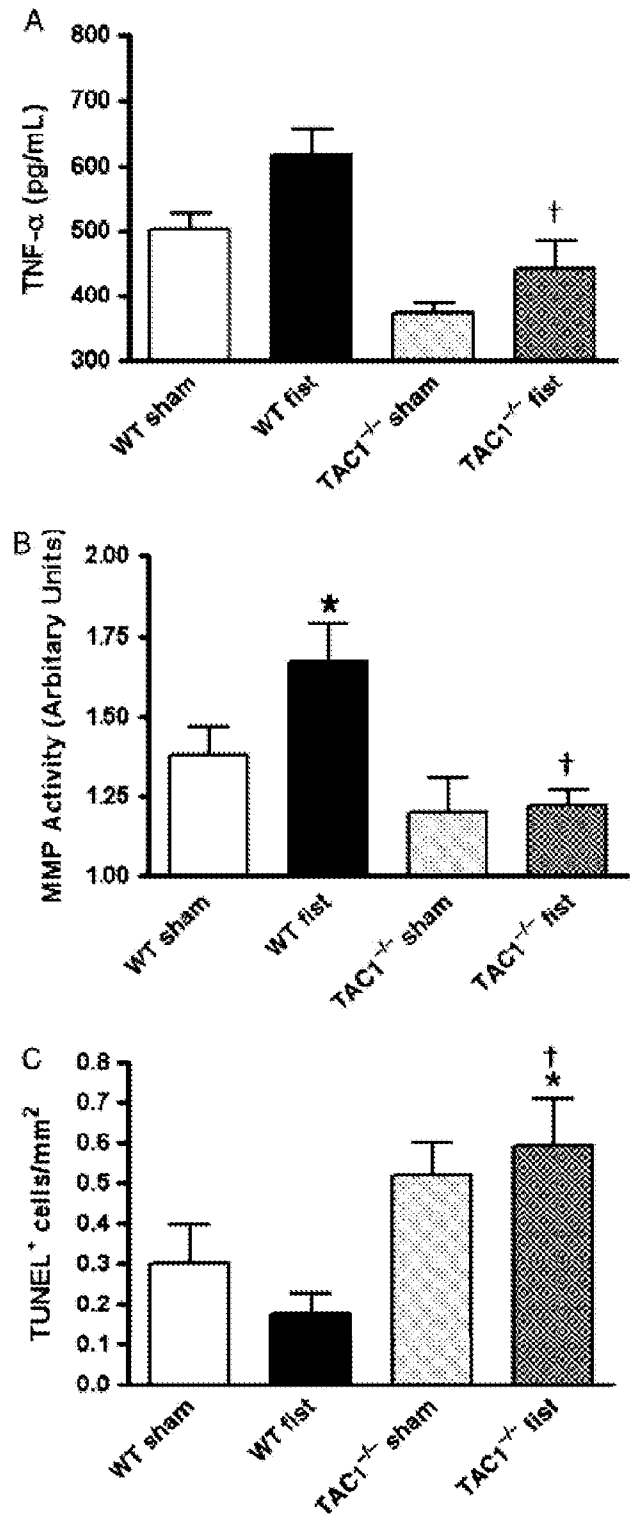


FIG. 2

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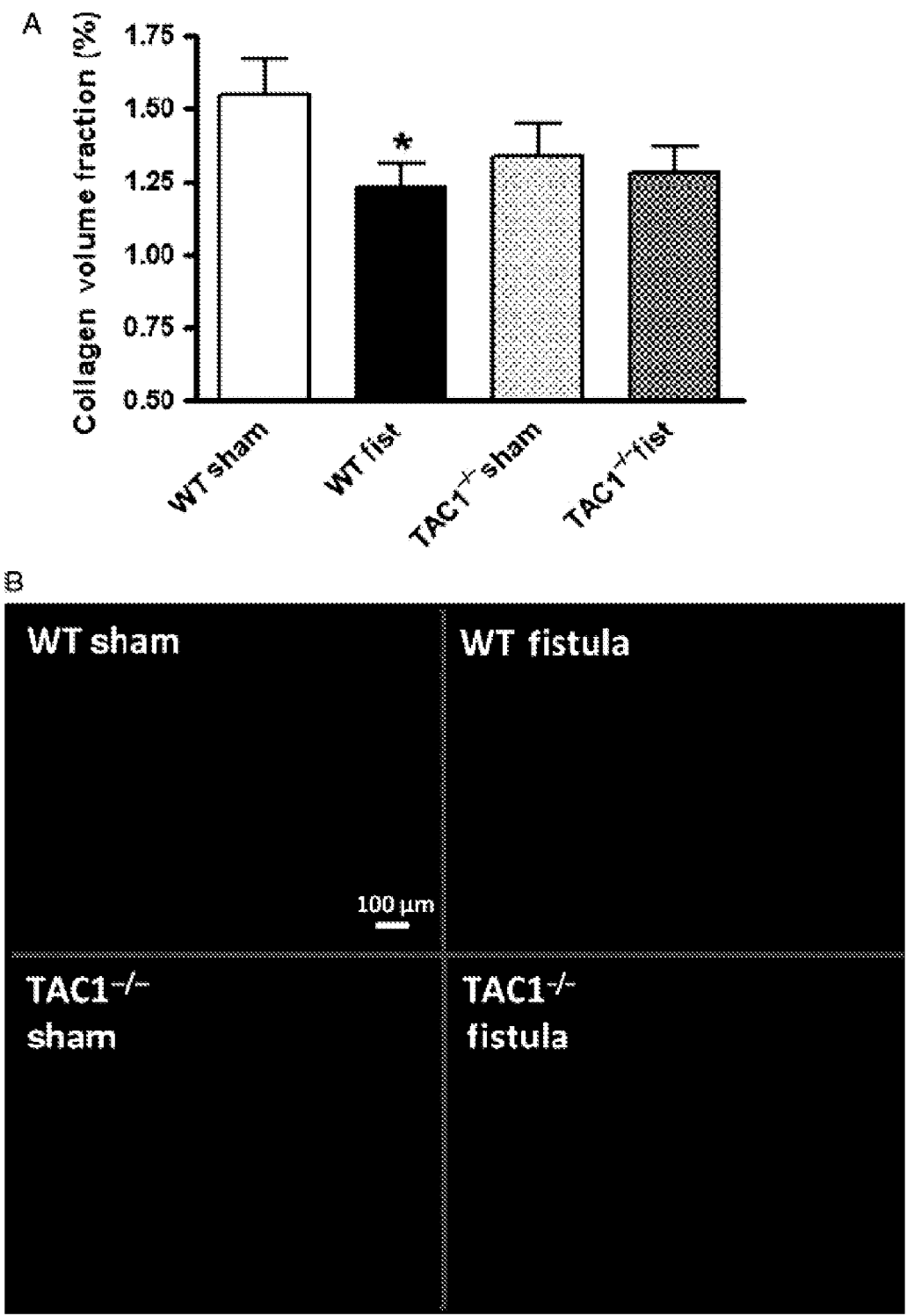


FIG. 3

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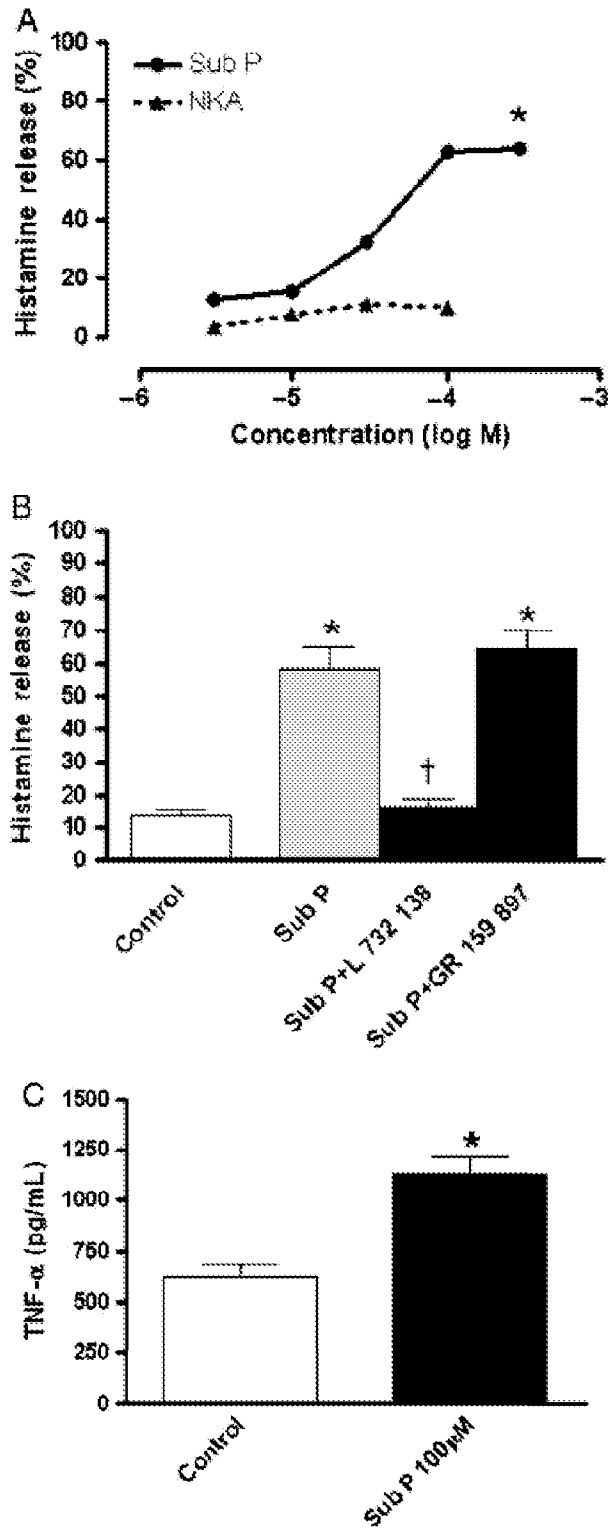


FIG. 4

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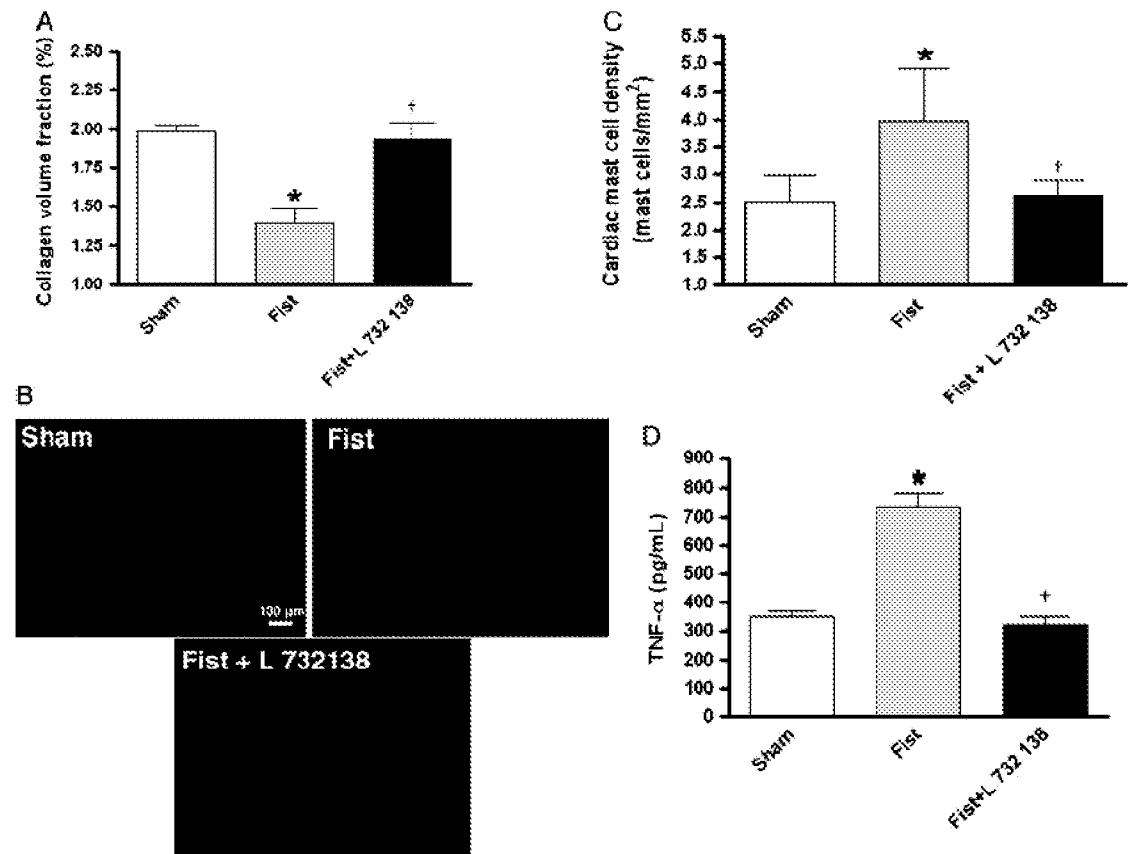
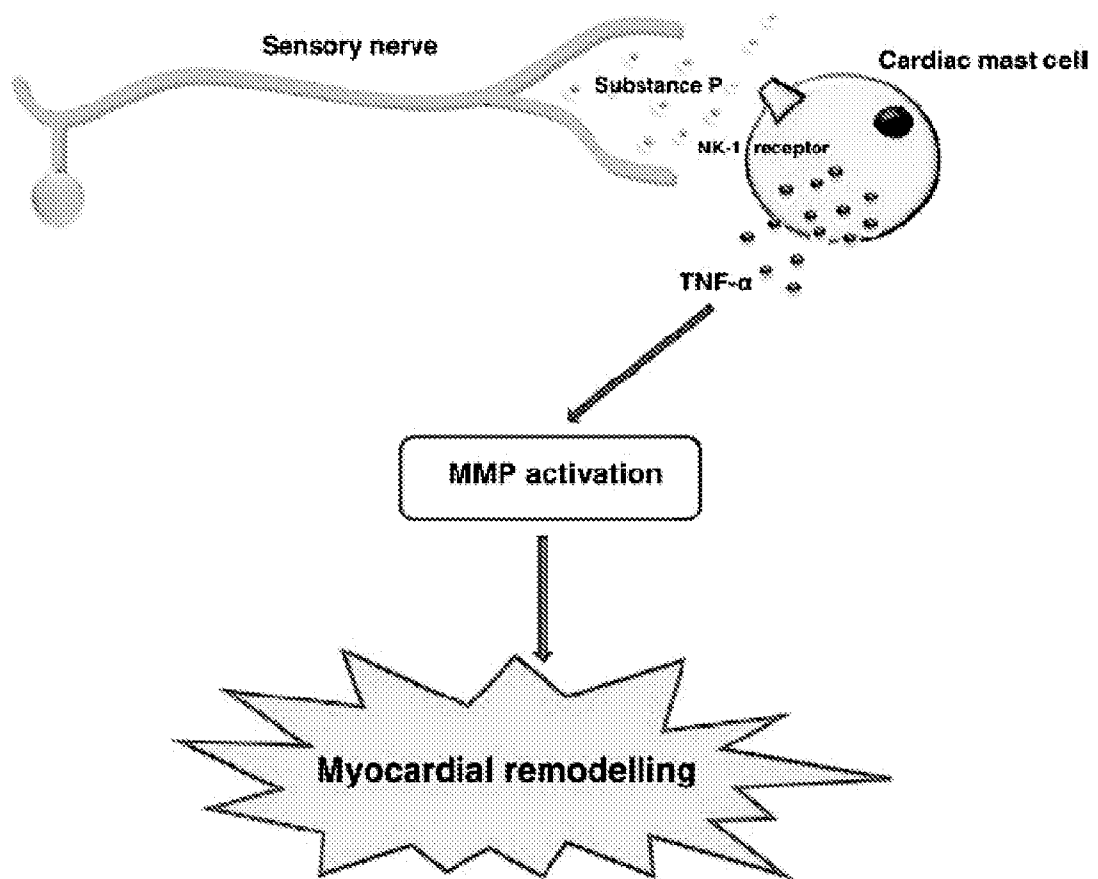


FIG. 5

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**FIG. 6**