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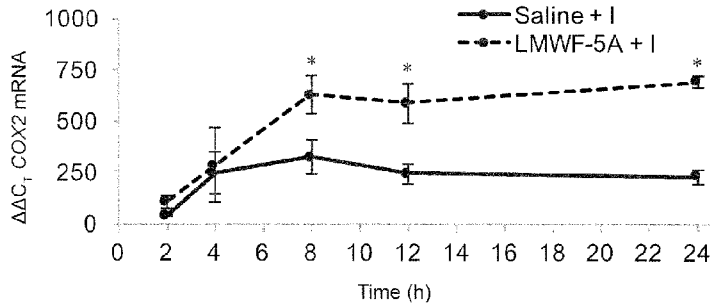
(54) Title: USE OF LOW MOLECULAR WEIGHT FRACTIONS OF HUMAN SERUM ALBUMIN IN TREATING DISEASES

Fig. 1A



(57) Abstract: The present invention provides a method of modulating various aspects of the immune system. In particular, the present invention teaches the use of diketopiperazines (DKPs) to modulate various aspects of the immune system such as, for example, inflammation, T-cells and various cytokines.

USE OF LOW MOLECULAR WEIGHT FRACTIONS OF HUMAN SERUM ALBUMIN IN TREATING DISEASES

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of priority under 35 U.S.C. §119(e) to U.S. Provisional Patent Application No. 62/182,985, filed June 22, 2015 and U.S. Provisional Patent Application No. 62/318,873, filed April 4, 2016. The entire disclosure of U.S. Provisional Patent Application No. 62/182,985 and U.S. Provisional Patent Application No. 62/318,873 are incorporated herein by reference.

FIELD OF THE INVENTION

This invention relates to the treatment of diseases using a low molecular weight fraction (LMWF) of human serum albumin (HSA) wherein at least one of its components comprises a diketopiperazine (DKP) having amino acid side chains of alanine and aspartic acid and referred to as DA-DKP. In particular, the present invention teaches the use of this LMWF of HSA to modulate various aspects of the immune system such as, for example, inflammation, T-cells and various cytokines.

BACKGROUND

The vertebrate immune system is comprised of subsystems that are classified depending on the type of immune response being mounted. These subsystems work together to mount an initial immune response following an infection or damage and to produce a more specific response to the infecting organism over time. For example, one such subsystem is the innate immune system (also referred to as the non-specific immune system). The innate immune system often referred to as the first line of defense because molecules and cells of the innate immune system mount a generic response to threats to the individual. That is, the innate immune system responds similarly to all damage and infections without regard to the specificity of the damaging or infectious agent. Inflammation is one of the first parts of the innate immunes system to respond to an insult. Inflammation is mediated by numerous chemical agents released by damaged or infected cells and serves to produce a physical barrier to further infection and also helps promote healing of damaged tissue. Examples of such agents include platelet activating growth factor (PAGF), which is a potent activator and mediator of inflammation, and interleukin-8 (IL-8;

neutrophil chemotactic factor), which induces migration of primary neutrophils to sites of infection and initiation of phagocytosis.

A second subsystem of the immune system is the adaptive immune system (acquired immune system, specific immune system). The adaptive immune system is comprised of immune molecules (e.g., cytokines) and highly specialized immune cells that evolve to recognize specific molecules from specific pathogens. Examples of cells that make up the adaptive immune system include T and B-cells.

T-cell mediated diseases represent a large number of immune system disorders. In particular, T-cells are thought to be the cells that start and perpetuate autoimmune diseases. Autoimmune diseases are a group of serious, chronic illnesses that afflict millions of people in the United States alone. Autoimmune diseases are characterized by reactivity of the immune system to endogenous (self) antigens. These immune responses to self antigens are maintained by the persistent or recurrent activation of self-reactive T-cells and, directly or indirectly, the self-reactive T-cells are responsible for the characteristic tissue injury and destruction seen in autoimmune diseases. Although many treatments for autoimmune diseases and other T-cell mediated diseases have been proposed, there is still a need for additional treatments.

While the overall immune system provides the organism protection from physical injury and infectious agents, lack of control of the system can result in damage to the organisms own tissue. For example, following physical damage to the organism, the non-specific nature of the innate immune response often results in damage to normal health tissue. Similarly, over or under-active T-cell responses can result in auto immune diseases such as arthritis, bursitis, allergies, asthma, sepsis, shock and the like. Thus, tight control of immune response initiation and inhibition are critical to maintain health and numerous compounds have been developed to establish and maintain such control (e.g., cyclooxygenase inhibitors such as aspirin, ibuprofen, etc.),

Diketopiperazines have been reported to exhibit a variety of biological activities. See, e.g., U.S. Patents Nos. 4,289,759 (immunoregulatory agents), 4,331,595 (immunoregulatory agents), 4,940,709 (PAF antagonists), 5,700,804 (inhibitors of plasminogen activator inhibitor), 5,750,530 (inhibitors of plasminogen activator inhibitor), 5,990,112 (inhibitors of metalloproteases), PCT applications WO 97/36888 (inhibitors of farnesyl-protein transferase) and WO 99/40931 (treatment of central nervous system injury), EP application 43219

(immunoregulatory agents), Japanese application 63 290868 (PAF antagonists), Japanese application 31 76478 (immunosuppressive agents), Shimazaki et al., Chem. Pharm. Bull., 35(8), 3527-3530 (1987) (PAF antagonists), Shimazaki et al., J. Med. Chem., 30, 1709-1711 (1987) (PAF antagonists), Shimazaki et al., Lipids, 26(12), 1175-1178 (1991) (PAF antagonists), Yoshida et al., Prog. Biochem. Pharmacol., 22, 68-80 (1988) (PAF antagonists), Alvarez et al., J. Antibiotics, 47(11), 1195-1201 (1994) (inhibitors of calpain), the complete disclosures of which are incorporated herein by reference.

Many diketopiperazines are known. For example, the diketopiperazine composed of aspartic acid and alanine (3-methyl-2,5-diketopiperazine-6-acetic acid; DA-DKP) is known. It has been reported to be formed as a result of the degradation of human albumin stored above 30°C. Chan et al., *Eur. J. Biochem.*, **227**, 524-528 (1995). Moreover, the use of such diketopiperazines for treating diseases by manipulation of various aspects of the immune system is also known. However, there is still a need for improved methods for regulating the immune system and treating diseases. The present invention provides such methods.

SUMMARY OF THE INVENTION

One embodiment of the invention relates to a method of inhibiting inflammation by administering to an individual in need thereof an effective amount of a pharmaceutical composition comprising a low molecular weight fraction (LMWF) of human serum albumin (HSA), wherein the individual is not administered a cyclooxygenase-2 (COX-2) antagonist within the period of time ranging from about one hour before to about 6 hours after administration of the pharmaceutical composition.

Another embodiment of the invention relates to a method of treating a T-cell mediated disease by administering to an individual in need thereof an effective amount of a pharmaceutical composition comprising a low molecular weight fraction (LMWF) of human serum albumin (HSA), wherein the individual is not administered a cyclooxygenase-2 (COX-2) antagonist within the period of time ranging from about one hour before to about 6 hours after administration of the pharmaceutical composition.

In one aspect, the T-cell mediated disease is graft rejection, graft versus host disease, an unwanted delayed-type hypersensitivity reaction, a T-cell mediated pulmonary disease, an autoimmune disease or an inflammatory disease.

In another aspect, the T-cell-mediated disease is selected form the group consisting of

multiple sclerosis, neuritis, polymyositis, psoriasis, vitiligo, Sjogren's syndrome, rheumatoid arthritis, Type 1 diabetes, autoimmune pancreatitis, inflammatory bowel diseases, Crohn's disease, ulcerative colitis, celiac disease, glomerulonephritis, scleroderma, sarcoidosis, autoimmune thyroid diseases, Hashimoto's thyroiditis, Graves disease, myasthenia gravis, Addison's disease, autoimmune uveoretinitis, pemphigus vulgaris, primary biliary cirrhosis, pernicious anemia and systemic lupus erythematosus.

In yet another aspect, the T-cell-mediated disease is pulmonary fibrosis or idiopathic pulmonary fibrosis.

In still another aspect, the T-cell-mediated disease is an inflammatory disease.

Yet another embodiment of the invention relates to a method of treating a joint disease or condition by administering to an individual in need thereof an effective amount of a pharmaceutical composition comprising a low molecular weight fraction (LMWF) of human serum albumin (HSA), wherein the individual is not administered a cyclooxygenase-2 (COX-2) antagonist within the period of time ranging from about one hour before to about 6 hours after administration of the pharmaceutical composition.

In one aspect, the joint disease or condition is a degenerative joint disease.

Another embodiment of the invention relates to a method of reducing the level of interleukin-8 (IL-8) in an individual by administering to an individual in need thereof an effective amount of a pharmaceutical composition comprising a low molecular weight fraction (LMWF) of human serum albumin (HSA), wherein the individual is not administered a cyclooxygenase-2 (COX-2) antagonist within the period of time ranging from about one hour before to about 6 hours after administration of the pharmaceutical composition.

In any of the embodiments of the invention described above or elsewhere herein, in one aspect, the LMWF of HSA contains components having a molecular weight less than 5000.

In any of the embodiments of the invention described above or elsewhere herein, in one aspect, the LMWF of HSA contains components having a molecular weight less than 3000.

In any of the embodiments of the invention described above or elsewhere herein, in one aspect, the LMWF of HSA comprises DA-DKP.

In any of the embodiments of the invention described above or elsewhere herein, in one aspect, the LMWF of HSA comprises one or more compounds selected from the group consisting of N-acetyl tryptophan and caprylic acid.

In any of the embodiments of the invention described above or elsewhere herein, in one aspect, the individual receiving the pharmaceutical composition is not administered a COX-2 antagonist within a time period selected from the group consisting of at least about one hour, at least about 12 hours, at least about one day, at least about two days, at least about three days, at least about four days, at least about five days, at least about six days, at least about seven days, at least about eight days or at least about nine days and 10 days, prior to administration of the pharmaceutical composition.

In any of the embodiments of the invention described above or elsewhere herein, in one aspect, the individual receiving the pharmaceutical composition is not administered a COX-2 antagonist during the period of time in which at least one active ingredient in the pharmaceutical composition exerts its effect. In one aspect, the composition that reduces COX-2 activity comprises a chemical selected from the group consisting of acetylsalicylic acid (aspirin), 2-(4-isobutylphenyl)propanoic acid (ibuprofen), *N*-(4-hydroxyphenyl)ethanamide (paracetamol), (S)-6-methoxy-α- methyl-2-naphthaleneacetic acid (naproxen), 2-[(2,6-dichlorophenyl)amino] benzeneacetic acid (diclofenac), 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl] benzenesulfonamide (celecoxib), 4-[4-(methylsulfonyl)phenyl]-3-phenyl-2(5H)-furanone (rofecoxib), and 4-(5-Methyl-3-phenylisoxazol-4-yl)benzolsulfonamid (valdecoxib).

In any of the embodiments of the invention described above or elsewhere herein, in one aspect, the individual receiving the pharmaceutical composition is not administered a COX-2 antagonist within a time period selected from the group consisting of at least 6 hours, at least 12 hours, at least about 24 hours, at least about two days, at least about three days, at least about four days, at least about five days, at least about six days, at last about one week, at least about two weeks, at least about three weeks, at least about one month, at least about two months, at least about three months, at least about four months at least about five months or at least about six months after administration of a pharmaceutical composition of the invention.

BRIEF DESCRIPTION OF DRAWINGS

Figures 1A-1C show that the LMWF of HSA wherein at least one component comprises DA-DKP (also referred to herein as "LMWF-5A") increases *COX2* mRNA in OA synoviocytes (primary synoviocytes isolated from the knee synovial membrane of patients with osteoarthritis (OA)) over a 24 h time course. OA synoviocytes were cultured in the presence of LMWF-5A or saline with 10 ng/mL IL-1β (Fig. 1A), LMWF-5A or saline with 10 ng/mL TNFα (Fig. 1B), or

LMWF-5A or saline alone (Fig. 1C) for up to 24 h. Total RNA was harvested 2, 4, 8, 12, and 24 hours post-treatment, and qPCR was performed to quantify the total *COX2* mRNA and 18S rRNA expression. Using the $\Delta\Delta C_T$ method, relative fold changes were quantified and normalized to untreated OA synoviocytes. The normalized fold-change (mean ± SEM) is shown. * indicates significantly increased *COX2* mRNA when compared to the saline control at that time point ($p<0.05$; n=4).

Figures 2A-2F show that the LMWF of HSA increases COX2 protein in OA synoviocytes when co-stimulated with pro-inflammatory cytokines. Western blots of protein lysates from OA synoviocytes were probed with antibodies against COX2 and the loading control α-Tubulin (Figs. 2A, 2C, 2E). Relative band densities were graphed over time (Figs.2B, 2D,2 F). OA synoviocytes were stimulated with 10 ng/mL IL-1β (A, B), 10 ng/mL TNFα (Figs. 2C, 2D), or left unstimulated (Figs. 2E, 2F) in the presence of saline or LMWF-5A over a 24 h time course, and protein lysates were prepared at 2, 4, 8, 12, and 24 h. The normalized COX2 protein level (mean ± SEM) is shown. * indicates significantly increased *COX2* mRNA when compared to the saline control at that time point ($p<0.05$; n=4).

Figures 3A-3B show that the LMWF of HSA affects prostaglandin release by cytokine-stimulated OA synoviocytes. Cell culture media was collected from OA synoviocyte cultures 24 h after stimulation with either 10 ng/mL IL-1β or TNFα in the presence of saline or LMWF-5A. PGE2 (Fig. 3A) and PGD2 (Fig. 3B) were quantified by competitive ELISA. The mean concentration ± SEM for four independent experiments were graphed, and * indicates a significant increase ($p<0.05$) in PG in the media when compared to the corresponding saline control.

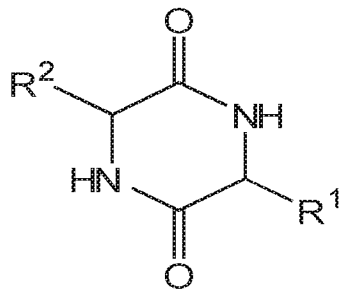
Figure 4: Proposed mechanism of action for the LMWF of HSA with respect to the COX2 pathway.

Figure 5 shows the percent change in LPS induced PMBC Release as discussed in Example 2.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a method of modulating various aspects of the immune system. In particular, the present invention teaches the use of a low molecular weight fraction of a solution of human serum albumin, which includes aspartic acid-alanine-diketopiperazine (DKP) to modulate various aspects of the immune system such as, for example, T-cells and various cytokines. Because the composition disclosed herein is capable of modulating aspects of the

immune system, it can therefore be used to treat various diseases and conditions. aspartic acid-alanine-diketopiperazine has Formula (I):



wherein:

R^1 and R^2 are different and each is the side chain of an amino acid selected from the group consisting of alanine and aspartic acid; or a physiologically-acceptable salt thereof. This diketopiperazine is referred to as DA-DKP (also referred to as aspartic acid-alanine-DKP or Asp-Ala-DKP or 3-methyl-2,5-diketopiperazine-6-acetic acid).

The present inventors have discovered that a low molecular weight fraction ("LMWF") of human serum albumin (HSA) functions by increasing levels of cyclooxygenase-2 (COX-2).

The term "LMWF" refers to a low molecular weight fraction of HSA that is a composition prepared by separation of high molecular weight components from human serum albumin (HSA). For example, LMWF can be prepared by filtration of a commercially available HSA solution wherein molecular weight components of more than 3 kilo daltons (kDa), 5 kDa, 10 kDa, 20 kDa, 30 kDa, 40 kDa, 50 kDa, are separated from the HSA solution. Alternatively, the term LMWF can refer to a composition prepared by separation of the high molecular weight components by other techniques, including but not limited to ultrafiltration, column chromatography including size exclusion chromatography, affinity chromatography, anion exchange, cation exchange, sucrose gradient centrifugation, salt precipitation, or sonication. LMWF also refers to a composition that includes components of HSA having a molecular weight less than 50,000 daltons (Da) (or 50 kDa), 40 kDa, 30 kDa, 20 kDa, 10 kDa, 5000 Da, 4000 Da, or 3000 Da (corresponding to 50,000 g/mol, 40,000 g/ml, 30,000 g/mol, 20,00 g/mol, 10,000 g/mol, 5,000 g/mol, 4,000 g/mol or 3,000 g/mol respectively).

At least one of the components in LMWF is DA-DKP.

In embodiments of the invention, individuals being treated with LMWF as disclosed herein do not receive any COX-2 antagonists prior or subsequently to administration of LMWF in a manner that a COX-2 antagonist does not interfere with the mechanism of action of LMWF which as has now been recognized by the inventor includes increasing levels of COX-2. Thus, a method of the present invention can generally be practiced by administering to an individual a composition comprising LMWF, wherein at the time of administration the individual's immune system is not being significantly affected by a COX-2 antagonist. That is, in the time period prior and/or subsequent to administration of the LMWF of HSA (e.g., 30 minutes, one hour, six hours, 12 hours, 24 hours, one day, one week, etc.) the individual being treated has not been administered, or has not self-administered, a compound that reduces or inhibitsCOX-2 activity.

DA-DKP is known to occur in solutions of human serum albumin and therefore, in LMWF of the present invention will occur because DA-DKP is a low molecular weight molecule. In certain embodiments, DA-DKP will be present in LMWF in concentrations ranging from about 0 μM DA-DKP to about 200 μM DA-DKP. In still other embodiments the DA-DKP will be present in LMWF in concentrations ranging from about 50 μM DA-DKP to about 100 μM DA-DKP. In addition, concentrations of DA-DKP in LMWF can be modified by addition of DA-DKP. For example, DA-DKP concentrations can be increased by methods described in U.S. Patent Publication No. 2015/0366932. Alternatively, DA-DKP can be made synthetically. Methods of preparing diketopiperazines, such as DA-DKP are known in the art, and such methods can be used to synthesize DA-DKP. See, e.g., See, *e.g.*, U.S. Patents Nos. 4,694,081, 5,817,751, 5,990,112, 5,932,579, U.S. Patent Publication No.2004/0024180, PCT Publication Nos. WO 96/00391 and WO 97/48645, and Smith et al., *Bioorg.* Med. Chem. Letters, 8, 2369-2374 (1998), the complete disclosures of which are incorporated herein by reference. In addition, DA-DKP can be synthesized by methods described in U.S. Patent Publication No. 2015/0366932.

In addition, the LMWF compositions of the present invention wherein at least one of the components of the LMWF comprises DA-DKP, will further comprise additional compounds. Examples of such compounds include, but are not limited to, N-acetyl tryptophan (NAT), caprylic acid, caprylate, or combinations thereof. The concentration of any one of N-acetyl tryptophan (NAT), caprylic acid, caprylate, or combinations thereof in the product can be in the range of about

4 mM to about 20 mM. The concentration of these components will be in the range of amounts that are found in commercial solutions of human serum albumin.

One embodiment of the present invention is a method of inhibiting inflammation, the method comprising administering to an individual in need thereof an effective amount of a pharmaceutical composition comprising a low molecular weight fraction (LMWF) of human serum albumin (HSA), wherein the individual is not administered a cyclooxygenase-2 (COX-2) antagonist within the period of time ranging from about one hour before to about 6 hours after administration of the pharmaceutical composition.

Another embodiment of the present invention is a method of treating a T-cell mediated disease, comprising administering to an individual in need thereof an effective amount of a pharmaceutical composition comprising a low molecular weight fraction (LMWF) of human serum albumin (HSA), wherein the individual is not administered a cyclooxygenase-2 (COX-2) antagonist within the period of time ranging from about one hour before to about 6 hours after administration of the pharmaceutical composition.

Another embodiment of the present invention is a method of preventing a T-cell mediated disease, comprising administering to an individual in need thereof an effective amount of a pharmaceutical composition comprising a low molecular weight fraction (LMWF) of human serum albumin (HSA), wherein the individual is not administered a cyclooxygenase-2 (COX-2) antagonist within the period of time ranging from about one hour before to about 6 hours after administration of the pharmaceutical composition.

As used herein, to treat means to reduce (wholly or partially) the symptoms, duration or severity of a disease, including curing the disease. In addition, the terms administering, administered, administer, and the like, are meant to encompass administration of a compound to an individual through self-administration or by another individual by any suitable method. For example, the term refers to contact of a compound with the individual being treated by, for example, methods such as oral ingestion, injection, infusion, application of a topical paste, nasally, rectally, vaginally, parenterally (*e.g.*, intra-articular, intravenously, intraspinally, intraperitoneally, subcutaneously, or intramuscularly), intracisternally, transdermally, intracranially, intracerebrally, and topically (including buccally and sublingually). The composition of the present invention comprising a LMWF of HSA may be administered to an individual by any suitable route of administration, including locally, parenterally (e.g., injection, intra-articular injection,

intravenously, intraspinally, intraperitoneally, subcutaneously, or intramuscularly), transdermally, and topically.

According to the present invention, T-cell mediated diseases are those resulting from an increase or decrease in the level of T-cells and/or T-cell activity. Examples of T-cell mediated diseases include, but are not limited to, graft rejection, graft versus host disease, unwanted delayed-type hypersensitivity reactions (such as delayed-type allergic reactions), T-cell mediated pulmonary diseases, and autoimmune diseases. T-cell mediated pulmonary diseases include sarcoidosis, hypersensitivity pneumonitis, acute interstitial pneumonitis, alveolitis, pulmonary fibrosis, idiopathic pulmonary fibrosis and other diseases characterized by inflammatory lung damage. Autoimmune diseases include multiple sclerosis, neuritis, polymyositis, psoriasis, vitiligo, Sjogren's syndrome, rheumatoid arthritis, Type 1 diabetes, autoimmune pancreatitis, inflammatory bowel diseases (*e.g.*, Crohn's disease and ulcerative colitis), celiac disease, glomerulonephritis, scleroderma, sarcoidosis, autoimmune thyroid diseases (*e.g.*, Hashimoto's thyroiditis and Graves disease), myasthenia gravis, Addison's disease, autoimmune uveoretinitis, pemphigus vulgaris, primary biliary cirrhosis, pernicious anemia, and systemic lupus erythematosis.

Pharmaceutical compositions useful for practicing the present invention can be administered in any form and by any method. Examples of useful routes of administration include, but are not limited to, orally, nasally, rectally, vaginally, parenterally (*e.g.*, intravenously, intraspinally, intraperitoneally, subcutaneously, or intramuscularly), intracisternally, transdermally, intracranially, intracerebrally, and topically (including buccally and sublingually). Preferred routes of administration are orally and intravenously. Examples of useful forms of pharmaceutical compositions and routes of administration are described in detail in U.S. Patent Nos. 8,183,209 and 8,980,834, the entirety of which are incorporated herein by reference.

In certain embodiments, the individual being treated is a mammal, such as a rabbit, goat, dog, cat, horse or human. In one embodiment, the individual is a human.

As has been described, for example in US 8,183,209, diketopiperazines for use in the present invention can be prepared by heating solutions of albumin. The solution can be a concentrated solution (e.g., about 100-500 mM) to achieve protonation of the N-terminal and/or C-terminal amino acid. The solution is heated at, for example, 60°C for from about 2 hours to several days, preferably about 4 days, to cause formation of the diketopiperazine. Thus, in one

embodiment, prior to passing the albumin solution over the filtration device, the albumin solution is heated under conditions effective to cause formation of diketopiperazines. In one embodiment, the albumin solution is heated to at least 60°C.

The diseases and conditions disclosed herein, are treated by administering to individual in need thereof an effective amount of a pharmaceutical composition comprising a low molecular weight fraction (LMWF) of human serum albumin (HSA). In addition, the individual is not administered a cyclooxygenase-2 (COX-2) antagonist during the time of administration of the LMWF, such as within the period of time ranging from about one hour before to about 6 hours after administration of the pharmaceutical composition.

As previously discussed, pharmaceutical compositions of the invention can comprise additional compounds. In one embodiment, the pharmaceutical composition comprises N-acetyl tryptophan (NAT). In one embodiment, the pharmaceutical composition comprises caprylic acid and/or caprylate. In one embodiment, the pharmaceutical composition comprises at least one compound selected from the group consisting of NAT, caprylic acid, caprylate and mixture thereof.

Because the biological activity of the LMWF of HSA wherein at least one of the components is DA-DKP, is due, at least in part, to an increase in COX-2 activity, the individual to which the pharmaceutical composition is being administered should not have been administered, or should not have self-administered, a compound that reduces COX-2 activity prior or subsequent to administration of the pharmaceutical composition in a manner that substantially affects the biological activity of the LMWF. As used herein, a compound that reduces COX-2 activity is one that causes a reduction in the level of COX-2 enzymatic activity of at least about 10%, at last about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at last about 90%, at least about 95% or about 100% compared to the level of COX-2 enzymatic activity observed from a COX-2 enzyme not exposed to the LMWF. According to the present invention, a compound that reduces COX-2 activity can be referred to as a COX-2 antagonist and include known COX-2 inhibitors. COX-2 antagonists are known in the art, examples of which include, but are not limited to, acetylsalicylic acid (aspirin), 2-(4-isobutylphenyl)propanoic acid (ibuprofen), N-(4-hydroxyphenyl)ethanamide (paracetamol), (S)-6-methoxy-α- methyl-2-naphthaleneacetic acid (naproxen), 2-[(2,6-dichlorophenyl)amino] benzeneacetic acid

(diclofenac), 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl] benzenesulfonamide (celecoxib), 4-[4-(methylsulfonyl)phenyl]-3-phenyl-2(5H)-furanone (rofecoxib), and 4-(5-Methyl-3-phenylisoxazol-4-yl)benzolsulfonamid (valdecoxib).

It is appreciated by those skilled in the art that COX-2 antagonist may be cleared from an individual's body at different rates. Such rates depend on the half-life, the time to steady state, the method of clearance, the species of the individual, and the like. For example, while naproxen can usually be cleared from the body in 4-5 days, complete clearance of aspirin can take 5 days. Of course, the time needed to eliminate a COX-2 antagonist to an insignificant level (i.e., the level at which no appreciable effect of the COX-2 antagonist on treatment with a pharmaceutical composition of the invention is observed) is affected by the size of the dose as well as whether one or multiple doses of the COX-2 antagonist were taken. In preferred embodiments, the time between when the final administration of a COX-2 antagonist (i.e., the last time a COX-2 antagonist was taken or administered) and the time of administration of a pharmaceutical composition of the invention is determined based on the pharmacokinetics of the particular COX-2 antagonist.

In one embodiment, the time period between final administration of a COX-2 antagonist and administration of a pharmaceutical composition of the invention is at least equal to the total clearance time (i.e., the time needed to reduce the level of the COX-2 antagonist in the individual being treated to an insignificant level) of the COX-2 antagonist. In one embodiment, the time period between final administration of a COX-2 antagonist and administration of a pharmaceutical composition of the invention is greater than the total clearance time of the COX-2 antagonist. In one embodiment, the time period between final administration of a COX-2 antagonist and administration of a pharmaceutical composition of the invention is at least 70%, at least 80%, at least 90%, or at least 95% of the total clearance time of the COX-2 antagonist.

In one embodiment, the individual receiving the pharmaceutical composition is not administered a COX-2 antagonist within a time period selected from the group consisting of at least about 15 minutes, at least about 30 minutes, at least about one hour, at least about two hours, at least about three hours, at least about four hours, at least about five hours, at least about six hours, at least about seven hours, at least about eight hours, at least about nine hours, at least about ten hours, at least about eleven hours, at least about twelve hours, at least about 13 hours, at least about 14 hours, at least about 15 hours, at least about 16 hours, at least about 17 hours, at least

about 18 hours, at least about 19 hours, at least about 20 hours, at least about 21 hours, at least about 22 hours, at least about 23 hours, at least about one day or 24 hours, at least about two days, at least about three days, at least about four days, at least about five days, at least about six days, at least about seven days, at least about eight days or at least about nine days and 10 days, prior to (or before) administration of the pharmaceutical composition. As used herein, the word about, with particular reference to time values, refers to a variation of 10%. According to the present disclosure, time periods for exclusion of administration of a COX-2 antagonist prior to treatment with a pharmaceutical composition of the invention can be defined in ranges using any of the times disclosed herein. For example, in one embodiment, the individual receiving the pharmaceutical composition has not been administered, or self-administered, a COX-2 antagonist in time period ranging from at least about 15 minutes to 10 days prior to administration of the pharmaceutical composition. In various embodiments of the invention, the individual receiving the pharmaceutical composition has not been administered, or self-administered, a COX-2 antagonist in time period ranging from at least about 15 minutes to at least about one day, at least about two days, at least about three days, at least about four days, at least about five days, at least about six days, at least about seven days, at least about eight days or at least about nine days prior to (or before) administration of the pharmaceutical composition. In still other embodiments of the invention, the individual receiving the pharmaceutical composition has not been administered, or self-administered, a COX-2 antagonist in time period ranging from at least about 30 minutes to at least about one day, at least about two days, at least about three days, at least about four days, at least about five days, at least about six days, at least about seven days, at least about eight days or at least about nine days prior to (or before) administration of the pharmaceutical composition. Examples of further ranges include, but are not limited to at least about 15 minutes to at least about 24 hours, 15 minutes to at least about two days, 15 minutes to at least about three days, 15 minutes to at least about four days, 15 minutes to at least about five days, 15 minutes to at least about six days, 15 minutes to at least about seven days, at least about 30 minutes, to at least about 4-5 days, at least about one hours to at least about 4-6 days, at least about 2-4 hours to about 4-6 days. The ranges disclosed herein are only meant as illustrative examples and are not meant to limit the invention to the specific ranges disclosed herein.

In addition to excluding administration of a COX-2 antagonist within a range of time prior to administration of a pharmaceutical composition of the invention, those skilled in the art will

understand that because the pharmaceutical composition of the invention has a range of time during which it is active, COX-2 antagonists should not be administered in a time period following (or after) administration of a pharmaceutical composition of the invention. Generally, this time period is the time period during which the active ingredient(s) of the pharmaceutical composition exert its/their effect. As used herein, an active ingredient is any ingredient present in the pharmaceutical composition that contributes to any of the biological effects disclosed herein (e.g., inhibiting T-cell activation, inhibiting inflammation, inhibiting PAF aggregation, etc.) For example, this time period can be the clearance time of the active ingredient(s) in the pharmaceutical composition. In one embodiment, the individual is not administered a COX-2 antagonist for at least about 2 hours, at least about 4 hours, at least about 6 hours, at least about 8 hours, at least about 10 hours, at least 12 hours, at least about 24 hours, at least about two days, at least about three days, at least about four days, at least about five days, at least about six days, at last about one week, at least about two weeks, at least about three weeks, at least about one month, at least about two months, at least about three months, at least about four months at least about five months or at least about six months after (or following) administration of a pharmaceutical composition of the invention. As noted above, time periods can also be stated in ranges comprising any of the times disclosed herein. For example, a time period during which an individual being treated can be excluded from administration of a COX-2 antagonist can include a period of time from 10 days prior to administration of a pharmaceutical composition of the invention to six months after administration of a pharmaceutical composition of the invention. In one embodiment, the individual being treated is not administered a COX2 antagonist during a time period ranging from 10 days prior to administration of a pharmaceutical composition of the invention to six months after administration of a pharmaceutical composition of the invention. Further examples of useful time periods include, but are not limited to a time period ranging from 10 days prior to administration of a pharmaceutical composition of the invention to six months after administration of a pharmaceutical composition of the invention; a time period ranging from 10 days prior to administration of a pharmaceutical composition of the invention to one week after administration of a pharmaceutical composition of the invention; a time period ranging from 10 days prior to administration of a pharmaceutical composition of the invention to two weeks after administration of a pharmaceutical composition of the invention; a time period ranging from 10 days prior to administration of a pharmaceutical composition of the invention to three weeks after

administration of a pharmaceutical composition of the invention; a time period ranging from 10 days prior to administration of a pharmaceutical composition of the invention to four weeks after administration of a pharmaceutical composition of the invention; a time period ranging from 10 days prior to administration of a pharmaceutical composition of the invention to two months after administration of a pharmaceutical composition of the invention; a time period ranging from seven days prior to administration of a pharmaceutical composition of the invention to one week after administration of a pharmaceutical composition of the invention; a time period ranging from seven days prior to administration of a pharmaceutical composition of the invention to two weeks after administration of a pharmaceutical composition of the invention; a time period ranging from seven days prior to administration of a pharmaceutical composition of the invention to three weeks after administration of a pharmaceutical composition of the invention; a time period ranging from seven days prior to administration of a pharmaceutical composition of the invention to one month after administration of a pharmaceutical composition of the invention. Such time ranges are meant as illustrative examples only and are not meant to limit the invention as other time ranges can be used based on the times disclosed herein.

The LMWF of HSA disclosed herein is effective in treating T-cell mediated diseases because it inhibits, among other things, the activation of T-cells. "Inhibit" is used herein to mean to reduce (wholly or partially). Thus, one embodiment of the present invention is a method of inhibiting T-cell activation in an individual, comprising administering to an individual an effective amount of a pharmaceutical composition comprising administering to an individual in need thereof an effective amount of a pharmaceutical composition comprising a low molecular weight fraction (LMWF) of human serum albumin (HSA), wherein the individual is not administered a cyclooxygenase-2 (COX-2) antagonist within the period of time ranging from about one hour before to about six hours after administration of the pharmaceutical composition. In one aspect, the LMWF of HSA contains components having a molecular weight of less than 5000. In another aspect, the LMWF of HSA contains components having a molecular weight of less than 3000. In a preferred aspect, at least one of the components in the LMWF comprises DA-DKP. In one embodiment, the individual receiving the pharmaceutical composition has not been administered a composition that reduces COX-2 activity in a time period selected from the group consisting of at least about one hour, at least about 12 hours, at least about one day, at least about two days, at least about three days, at least about four days, at least about five days, at least about six days, at

least about seven days, at least about eight days or at least about nine days and 10 days, prior to administration of the pharmaceutical composition. In still another aspect, the pharmaceutical composition further comprises at least one compound selected from the group consisting of NAT, caprylic acid, caprylate and mixtures thereof.

Because inflammation is exacerbated by, or involves, activated T-cells, the LMWF of HSA of the invention can be used to treat inflammation and inflammatory diseases and/or to prevent inflammation and inflammatory diseases. Thus, one embodiment of the present invention is a method of reducing inflammation in an individual, comprising administering to the individual an effective amount of a pharmaceutical composition comprising administering to an individual in need thereof an effective amount of a pharmaceutical composition comprising a low molecular weight fraction (LMWF) of human serum albumin (HSA), wherein the individual is not administered a cyclooxygenase-2 (COX-2) antagonist within the period of time ranging from about one hour before to about six hours after administration of the pharmaceutical composition. In one aspect, the LMWF of HSA contains components having a molecular weight of less than 5000. In another aspect, the LMWF of HSA contains components having a molecular weight of less than 3000. In a preferred aspect, at least one of the components in the LMWF comprises DA-DKP. In one embodiment, the individual receiving the pharmaceutical composition has not been administered a composition that reduces COX-2 activity in a time period selected from the group consisting of at least about one hour, at least about 12 hours, at least about one day, at least about two days, at least about three days, at least about four days, at least about five days, at least about six days, at least about seven days, at least about eight days or at least about nine days and 10 days, prior to administration of the pharmaceutical composition. In still another aspect, the pharmaceutical composition further comprises at least one compound selected from the group consisting of NAT, caprylic acid, caprylate and mixtures thereof.

One embodiment of the present invention is a method of reducing the severity and/or symptoms of multiple sclerosis comprising administering to an individual in need thereof an effective amount of a pharmaceutical composition comprising a low molecular weight fraction (LMWF) of human serum albumin (HSA), wherein the individual is not administered a cyclooxygenase-2 (COX-2) antagonist within the period of time ranging from about one hour before to about six hours after administration of the pharmaceutical composition. In one aspect, the LMWF of HSA contains components having a molecular weight of less than 5000. In another

aspect, the LMWF of HSA contains components having a molecular weight of less than 3000. In a preferred aspect, at least one of the components in the LMWF comprises DA-DKP. In one embodiment, the individual receiving the pharmaceutical composition has not been administered a composition that reduces COX-2 activity in a time period selected from the group consisting of at least about one hour, at least about 12 hours, at least about one day, at least about two days, at least about three days, at least about four days, at least about five days, at least about six days, at least about seven days, at least about eight days or at least about nine days and 10 days, prior to administration of the pharmaceutical composition. In still another aspect, the pharmaceutical composition further comprises at least one compound selected from the group consisting of NAT, caprylic acid, caprylate and mixtures thereof.

Diketopiperazines, such as DA-DKP, have also been shown to inhibit platelet activating factor. (see, for example, U.S. Patent Nos. 6,555,543, 8,455,517, 8,440,696 and 8,841,307, the disclosures of which are incorporated herein by reference). Platelet activating factor (PAF; 1-O-alkyl-2-acetyl-sn-glycerol-3-phosphorylcholine) is a potent inflammatory phospholipid mediator with a wide variety of biological activities. It is generated and released by basophils, monocytes, macrophages, polymorphonuclear leukocytes, eosinophils, neutrophils, natural killer lymphocytes, platelets and endothelial cells, as well as by renal and cardiac tissues under appropriate immunological and non-immunological stimulation. PAF mediates biological responses by binding to specific PAF receptors found in a wide variety of cells and tissues.

PAF also appears to play a role in pathological immune and inflammatory responses. Many published studies have provided evidence for the involvement of PAF in diseases, including arthritis, acute inflammation, asthma, allergic reactions, cardiovascular diseases, neoplastic diseases, endotoxic shock, pain, psoriasis, ophthalmic inflammation, ischemia, gastrointestinal ulceration, myocardial infarction, inflammatory bowel diseases, and acute respiratory distress syndrome. (See, for example, PCT application WO 94/04537 and US 6,555,543).

Accordingly, one embodiment of the present invention is a method of inhibiting PAF in an individual, comprising administering to an individual in need thereof an effective amount of a pharmaceutical composition comprising a low molecular weight fraction (LMWF) of human serum albumin (HSA), wherein the individual is not administered a cyclooxygenase-2 (COX-2) antagonist within the period of time ranging from about one hour before to about six hours after administration of the pharmaceutical composition. In one aspect, the LMWF of HSA contains

components having a molecular weight of less than 5000. In another aspect, the LMWF of HSA contains components having a molecular weight of less than 3000. In a preferred aspect, at least one of the components in the LMWF comprises DA-DKP. In one embodiment, the individual receiving the pharmaceutical composition has not been administered a composition that reduces COX-2 activity in a time period selected from the group consisting of at least about one hour, at least about 12 hours, at least about one day, at least about two days, at least about three days, at least about four days, at least about five days, at least about six days, at least about seven days, at least about eight days or at least about nine days and 10 days, prior to administration of the pharmaceutical composition. In still another aspect, the pharmaceutical composition further comprises at least one compound selected from the group consisting of NAT, caprylic acid, caprylate and mixtures thereof.

PAF has been reported to play a role in a variety of disease and conditions. Examples of such diseases and conditions include acute respiratory distress syndrome, allergies, arthritis, asthma, autoimmune diseases, bronchitis, cardiovascular disease, Crohn's disease, cystic fibrosis, emphysema, gastrointestinal ulceration, inflammation, inflammatory bowel disease, ischemia, multiple organ dysfunction syndrome, myocardial infarction, neoplastic diseases, ophthalmic inflammation, pain, psoriasis, respiratory infections, sepsis, shock, and ulcerative colitis. Thus, one embodiment of the present invention is a method of treating a disease or condition mediated by PAF, the method, comprising administering to an individual in need of such treatment an effective amount of a pharmaceutical composition comprising administering to an individual in need thereof an effective amount of a pharmaceutical composition comprising a low molecular weight fraction (LMWF) of human serum albumin (HSA), wherein the individual is not administered a cyclooxygenase-2 (COX-2) antagonist within the period of time ranging from about one hour before to about six hours after administration of the pharmaceutical composition. In one aspect, the LMWF of HSA contains components having a molecular weight of less than 5000. In another aspect, the LMWF of HSA contains components having a molecular weight of less than 3000. In a preferred aspect, at least one of the components in the LMWF comprises DA-DKP. In one embodiment, the individual receiving the pharmaceutical composition has not been administered a composition that reduces COX-2 activity in a time period selected from the group consisting of at least about one hour, at least about 12 hours, at least about one day, at least about two days, at least about three days, at least about four days, at least about five days, at least about

six days, at least about seven days, at least about eight days or at least about nine days and 10 days, prior to administration of the pharmaceutical composition. In still another aspect, the pharmaceutical composition further comprises at least one compound selected from the group consisting of NAT, caprylic acid, caprylate and mixtures thereof. In one embodiment, the diseases is selected from the group consisting of acute respiratory distress syndrome, allergies, arthritis, asthma, autoimmune diseases, bronchitis, cardiovascular disease, Crohn's disease, cystic fibrosis, emphysema, gastrointestinal ulceration, inflammation, inflammatory bowel disease, ischemia, multiple organ dysfunction syndrome, myocardial infarction, neoplastic diseases, ophthalmic inflammation, pain, psoriasis, respiratory infections, sepsis, shock, and ulcerative colitis.

PAF also mediates platelet aggregation. Thus, one embodiment of the present invention is a method of inhibiting platelet aggregation in an individual, the method, comprising administering to an individual in need thereof an effective amount of a pharmaceutical composition comprising a low molecular weight fraction (LMWF) of human serum albumin (HSA), wherein the individual is not administered a cyclooxygenase-2 (COX-2) antagonist within the period of time ranging from about one hour before to about six hours after administration of the pharmaceutical composition. In one aspect, the LMWF of HSA contains components having a molecular weight of less than 5000. In another aspect, the LMWF of HSA contains components having a molecular weight of less than 3000. In a preferred aspect, at least one of the components in the LMWF comprises DA-DKP. In one embodiment, the individual receiving the pharmaceutical composition has not been administered a composition that reduces COX-2 activity in a time period selected from the group consisting of at least about one hour, at least about 12 hours, at least about one day, at least about two days, at least about three days, at least about four days, at least about five days, at least about six days, at least about seven days, at least about eight days or at least about nine days and 10 days, prior to administration of the pharmaceutical composition. In still another aspect, the pharmaceutical composition further comprises at least one compound selected from the group consisting of NAT, caprylic acid, caprylate and mixtures thereof.

One embodiment of the present invention is a method of reducing the level of IL-8 in an individual, the method, comprising administering to an individual in need thereof an effective amount of a pharmaceutical composition comprising a low molecular weight fraction (LMWF) of human serum albumin (HSA), wherein the individual is not administered a cyclooxygenase-2

(COX-2) antagonist within the period of time ranging from about one hour before to about six hours after administration of the pharmaceutical composition. In one aspect, the LMWF of HSA contains components having a molecular weight of less than 5000. In another aspect, the LMWF of HSA contains components having a molecular weight of less than 3000. In a preferred aspect, at least one of the components in the LMWF comprises DA-DKP. In one embodiment, the individual receiving the pharmaceutical composition has not been administered a composition that reduces COX-2 activity in a time period selected from the group consisting of at least about one hour, at least about 12 hours, at least about one day, at least about two days, at least about three days, at least about four days, at least about five days, at least about six days, at least about seven days, at least about eight days or at least about nine days and 10 days, prior to administration of the pharmaceutical composition. In still another aspect, the pharmaceutical composition further comprises at least one compound selected from the group consisting of NAT, caprylic acid, caprylate and mixtures thereof.

One embodiment of the present invention is a method of altering the level of PGE_2, PGI_1, $PGF_{2\alpha}$, PGD_2, 15d-PGJ_2, PPAR-γ, Ras, Erk, or a pathway affected thereby, in an individual, the method comprising administering to the individual an effective amount of a pharmaceutical composition comprising administering to an individual in need thereof an effective amount of a pharmaceutical composition comprising a low molecular weight fraction (LMWF) of human serum albumin (HSA), wherein the individual is not administered a cyclooxygenase-2 (COX-2) antagonist within the period of time ranging from about one hour before to about six hours after administration of the pharmaceutical composition. In one embodiment, the level of PGE_2, PGI_1, $PGF_{2\alpha}$, PGD_2, 15d-PGJ_2, PPAR-γ, Ras or Erk, is increased. In one embodiment, the level of PGE_2, PGI_1, $PGF_{2\alpha}$, PGD_2, 15d-PGJ_2, PPAR-γ, Ras or Erk, is decreased. In one aspect, the LMWF of HSA contains components having a molecular weight of less than 5000. In another aspect, the LMWF of HSA contains components having a molecular weight of less than 3000. In a preferred aspect, at least one of the components in the LMWF comprises DA-DKP. In one embodiment, the individual receiving the pharmaceutical composition has not been administered a composition that reduces COX-2 activity in a time period selected from the group consisting of at least about one hour, at least about 12 hours, at least about one day, at least about two days, at least about three days, at least about four days, at least about five days, at least about six days, at least about seven days, at least about eight days or at least about nine days and 10 days, prior to

administration of the pharmaceutical composition. In still another aspect, the pharmaceutical composition further comprises at least one compound selected from the group consisting of NAT, caprylic acid, caprylate and mixtures thereof.

PAF has been reported to induce the production and secretion of interleukin 8 (IL-8). IL-8 is a pro-inflammatory cytokine which has been reported to play a role in the pathogenesis of a large number of diseases and conditions, including acute respiratory distress syndrome, allergies, arthritis, asthma, autoimmune diseases, bronchitis, cancer, Crohn's disease, cystic fibrosis, emphysema, endocarditis, gastritis, inflammatory bowel disease, ischemia reperfusion, multiple organ dysfunction syndrome, nephritis, pancreatitis, respiratory viral infections, sepsis, shock, ulcerative colitis, and other inflammatory disorders. One embodiment of the present invention is a method of treating an IL-8-mediated disease in an individual, the method, comprising administering to the individual an effective amount of a pharmaceutical composition comprising administering to an individual in need thereof an effective amount of a pharmaceutical composition comprising a low molecular weight fraction (LMWF) of human serum albumin (HSA), wherein the individual is not administered a cyclooxygenase-2 (COX-2) antagonist within the period of time ranging from about one hour before to about six hours after administration of the pharmaceutical composition. As used herein, an IL-8-mediated disease is one in which the signs and/or symptoms result, at least in part, from the action of IL-8. In one aspect, the LMWF of HSA contains components having a molecular weight of less than 5000. In another aspect, the LMWF of HSA contains components having a molecular weight of less than 3000. In a preferred aspect, at least one of the components in the LMWF comprises DA-DKP. In one embodiment, the individual receiving the pharmaceutical composition has not been administered a composition that reduces COX-2 activity in a time period selected from the group consisting of at least about one hour, at least about 12 hours, at least about one day, at least about two days, at least about three days, at least about four days, at least about five days, at least about six days, at least about seven days, at least about eight days or at least about nine days and 10 days, prior to administration of the pharmaceutical composition. In still another aspect, the pharmaceutical composition further comprises at least one compound selected from the group consisting of NAT, caprylic acid, caprylate and mixtures thereof.

Compositions of the invention can also be used to treat joint conditions. Examples of such treatment have been shown, for example, in U.S. Patent No. 8,980,834, the entirety of which is

incorporated herein by reference. Thus, one embodiment of the present invention is a method of treating a joint condition in an individual, the method comprising administering to the individual an effective amount of a pharmaceutical composition comprising administering to an individual in need thereof an effective amount of a pharmaceutical composition comprising a low molecular weight fraction (LMWF) of human serum albumin (HSA), wherein the individual is not administered a cyclooxygenase-2 (COX-2) antagonist within the period of time ranging from about one hour before to about six hours after administration of the pharmaceutical composition. In one aspect, the LMWF of HSA contains components having a molecular weight of less than 5000. In another aspect, the LMWF of HSA contains components having a molecular weight of less than 3000. In a preferred aspect, at least one of the components in the LMWF comprises DA-DKP. In one embodiment, the individual receiving the pharmaceutical composition has not been administered a composition that reduces COX-2 activity in a time period selected from the group consisting of at least about one hour, at least about 12 hours, at least about one day, at least about two days, at least about three days, at least about four days, at least about five days, at least about six days, at least about seven days, at least about eight days or at least about nine days and 10 days, prior to administration of the pharmaceutical composition. In still another aspect, the pharmaceutical composition further comprises at least one compound selected from the group consisting of NAT, caprylic acid, caprylate and mixtures thereof.

As used herein, a joint condition can include one or more of inflammation, T-cells, B-cells, cytokine production, edema, pyrexia, pain and the like. Examples of joint conditions treatable using compositions of the present invention include, but are not limited to, ankylosing spondylitis, Behcet's syndrome, arthritis, rheumatoid arthritis, osteoarthritis, psoriatic arthritis and swelling due to trauma (e.g., dislocation, fracture, etc.) Any joint can be treated using a composition of the invention. Examples include, but are not limited to, toe joints, knee joints, hip joints, spinal joints, finger joints, hand joints, wrist joints, elbow joints, shoulder joints and neck joints. Further, compositions of the invention can be administered using any route of administration resulting in delivery of the composition into the joint. For example, in one embodiment a composition of the invention is injected directly into the synovial cavity (intra-articular injection).

One specific example of a joint condition is degenerative joint disease. A degenerative joint disease is a gradual deterioration of the articular cartilage that covers joints. A degenerative joint disease is a noninfectious progressive disorder of the weightbearing joints. The normal

articular joint cartilage is smooth, white, and translucent. It is composed of cartilage cells (chondrocytes) imbedded in a sponge-like matrix made of collagen, protein polysaccharides, and water. With early primary arthritis, the cartilage becomes yellow and opaque with localized areas of softening and roughening of the surfaces. As degeneration progresses, the soft areas become cracked and worn, exposing bone under the cartilage. The bone then begins to remodel and increase in density while any remaining cartilage begins to fray. Eventually, osteophytes (spurs of new bone) covered by cartilage form at the edge of the joint. As mechanical wear increases, the cartilage needs repairing. The cartilage cells are unable to produce enough of the sponge-like matrix and therefore the damaged cartilage cannot repair itself. The cartilage has no blood supply to enhance healing. The majority of degenerative joint disease is the result of mechanical instabilities or aging changes within the joint. This includes old age degenerative arthritis and, in younger individuals, may be the result of injuries, bruises, abnormal joint configuration (i.e. hip dysplasia), or mechanical wear from anterior cruciate ligament rupture, patellar luxation, or osteochondritis dissecans, for example. One embodiment of the present invention is a method of treating degenerative joint disease in an individual, the method comprising administering to the individual an effective amount of a pharmaceutical composition comprising administering to an individual in need thereof an effective amount of a pharmaceutical composition comprising a low molecular weight fraction (LMWF) of human serum albumin (HSA), wherein the individual is not administered a cyclooxygenase-2 (COX-2) antagonist within the period of time ranging from about one hour before to about six hours after administration of the pharmaceutical composition In one aspect, the LMWF of HSA contains components having a molecular weight of less than 5000. In another aspect, the LMWF of HSA contains components having a molecular weight of less than 3000. In a preferred aspect, at least one of the components in the LMWF comprises DA-DKP. In one embodiment, the individual receiving the pharmaceutical composition has not been administered a composition that reduces COX-2 activity in a time period selected from the group consisting of at least about one hour, at least about 12 hours, at least about one day, at least about two days, at least about three days, at least about four days, at least about five days, at least about six days, at least about seven days, at least about eight days or at least about nine days and 10 days, prior to administration of the pharmaceutical composition. In still another aspect, the pharmaceutical composition further comprises at least one compound selected from the group consisting of NAT, caprylic acid, caprylate and mixtures thereof.

While surgery on joint a joint usually results in repair of an underlying problem, the physical trauma of the surgery itself usually causes swelling of the joint accompanied by an immune response. Pharmaceutical compositions can be used to treat joints following surgery. One embodiment of the present invention is a method of reducing a post-surgical immune response, post-surgical swelling and/or post-surgical pain in a joint, the method comprising administering to the individual an effective amount of a pharmaceutical composition comprising administering to an individual in need thereof an effective amount of a pharmaceutical composition comprising a low molecular weight fraction (LMWF) of human serum albumin (HSA), wherein the individual is not administered a cyclooxygenase-2 (COX-2) antagonist within the period of time ranging from about one hour before to about six hours after administration of the pharmaceutical composition. In one aspect, the LMWF of HSA contains components having a molecular weight of less than 5000. In another aspect, the LMWF of HSA contains components having a molecular weight of less than 3000. In one embodiment, the individual receiving the pharmaceutical composition has not been administered a composition that reduces COX-2 activity in a time period selected from the group consisting of at least about one hour, at least about 12 hours, at least about one day, at least about two days, at least about three days, at least about four days, at least about five days, at least about six days, at least about seven days, at least about eight days or at least about nine days and 10 days, prior to administration of the pharmaceutical composition. In still another aspect, the pharmaceutical composition further comprises at least one compound selected from the group consisting of NAT, caprylic acid, caprylate and mixtures thereof.

It should be understood that treatment of a joint for an immune response, swelling, etc., due to surgical trauma can be administered to the individual undergoing surgery before or immediately after surgery. Moreover, it should be understood that the pharmaceutical composition can be administered starting before surgery and continuing on a time course following surgery. In one embodiment, the pharmaceutical composition is administered prior to surgery. In one embodiment, the pharmaceutical composition is administered starting at about 3 days, about two days, about one day, about twelve hours, about six hours or about three hours prior to surgery. In one embodiment, the pharmaceutical composition is administered for at least about one day, for at least about two days, for at least three days about 3 days, for at least about four days, for at least about five days, for at least about six days, for at least about one week, for at least about two weeks, for at least about three weeks or for at least about four weeks following surgery.

Because the treatment of the present invention provides a long-lasting effect on the symptoms of degenerative joint disease, one aspect of the present invention is that a pharmaceutical composition of the invention can be administered to an individual at longer time intervals than would be expected for conventional therapies, wherein the individual has not been administered a compound that reduces COX-2 activity in the at least ten, the at least nine, the at least eight, the at least seven days, the at least six days, the at least five days, the at least four days, the at least three days, the at least two days, the at least 24 hours, the at least 12 hours, the at least six hours, the at least five hours, the at least four hours, the at least three hours, the at least two hours, the at least one hour, the at least 30 minutes, the at least 15 minutes and the at least ten minutes prior to administration of the pharmaceutical composition. In one embodiment, a pharmaceutical composition of the invention can be administered to an individual at longer time intervals than would be expected for conventional therapies, wherein the pharmaceutical composition further comprises at least one compound selected from the group consisting of NAT, caprylic acid, caprylate and mixtures thereof, and wherein the individual has not been administered a compound that reduces the activity of cyclooxygenase-2 (COX-2) in the at least 15 minutes prior to administration of the pharmaceutical composition. For example, the present composition can be administered no more frequently than once every six months, once every five months, once every four months, once every three months, once every two months, once every month, once every four weeks, once every three weeks, once every two weeks or once every week.

It will be appreciated by those skilled in the art that because a pharmaceutical composition of the invention can be used in multiple treatments and for extended treatments of a disease or condition, in some embodiments the patient to which the pharmaceutical composition has been administered should not be administered a COX-2 antagonist for some period do time following administration of the pharmaceutical composition. The exact period of time will depend on the individual patent's ability to clear various compounds, including the compounds in a pharmaceutical composition of the invention e.g., LMWF of HSA, caprylate, N-acetyltryptophan). In one embodiment, a patient administered a pharmaceutical composition of the invention is not administered a COX-2 antagonist for at least one hour, at least two hours, at least three hours, at least four hours, at least five hours, at least six hours, at least 12 hours, at least one day, at least two days, at least three days, at least four days, at least five days, at least six days, at least one week, at least two weeks, at least three weeks or at least one month

following administration of the pharmaceutical composition.

The composition of the present invention may be a pharmaceutical solution having a LMWF of HSA wherein at least one component of the LMWF comprises DA-DKP wherein the DA-DKP concentration range with a lower endpoint of about 10 μM, about 20 μM, about 30 μM, about 40 μM, about 50 μM, about 60 μM, about 70 μM, about 80 μM, about 90 μM, about 100 μM, about 110 μM, about 120 μM, about 130 μM, about 140 μM, about 150 μM, about 160 μM, about 170 μM, about 180 μM, about 190 μM, about 200 μM, about 210 μM, about 220 μM, about 230 μM, about 240 μM, about 240, about 250 μM, about 260 μM, about 270 μM, about 280 μM, about 290 μM, about 300 μM, about 310, about 320 μM, about 330 μM, about 340 μM, about 350 μM, about 360 μM, about 370 μM, about 380 μM, about 390 μM, or about 400 μM. The composition of the present invention may be a pharmaceutical solution having a DA-DKP concentration range with an upper endpoint of about 600 μM, about 580 μM, about 570 μM, about 560 μM, about 550 μM, about 540 μM, about 530 μM, about 520 μM, about 510 μM, about 500 μM, about 490 μM, about 480 μM, about 470 μM, about 460 μM, about 450 μM, about 440 μM, about 430 μM, about 420 μM, about 410 μM, about 400 μM, about 390 μM, about 380 μM, about 370 μM, about 360 μM, about 350, about 340 μM, about 330 μM, about 320 μM, about 310 μM, about 300 μM, about 290 μM, about 280, about 270 μM, about 260 μM, about 250 μM, about 240 μM, about 230 μM, about 220 μM, about 210 μM, or about 200 μM.

An effective amount of the DA-DKP in the LMWF of HSA in the composition of the present invention for treating a degenerative joint disease or condition can be a range with a lower endpoint of about 10 μg, about 15 μg, about 20 μg, about 25 μg, about 30 μg, about 35 μg, about 40 μg, about 45 μg, about 50 μg, about 55 μg, about 60 μg, about 65 μg, about 70 μg, about 75 μg, about 80 μg, about 85 μg, about 90 μg, about 95 μg, about 100 μg, about 110 μg, about 120 μg, about 130 μg, about 140 μg, about 150 μg, about 160 μg, about 170 μg, about 180 μg, about 190 μg, about 200 μg, about 210 μg, about 220 μg, about 230 μg, about 240 μg, about 250 μg, about 260 μg, about 270 μg, about 280 μg, about 290 μg, about 300 μg, about 310 μg, about 320 μg, about 330 μg, about 340 μg, about 350 μg, about 360 μg, about 370 μg, about 380 μg, about 390 μg, about 400 μg, about 425 μg, about 450 μg, about 475 μg or about 500 μg. In addition, an effective amount of DA-DKP in the composition of the present invention for treating a degenerative joint disease or condition can be a range with upper endpoint of about 500 μg, about 490 μg, about 480 μg, about 470 μg, about 460 μg, about 450 μg, about 440 μg, about 430 μg,

about 420 μg, about 410 μg, about 400 μg, about 390 μg, about 380 μg, about 370 μg, about 360 μg, about 350 μg, about 340 μg, about 330 μg, about 320 μg, about 310 μg, about 300 μg, about 290 μg, about 280 μg, about 270 μg, about 260 μg, about 250 μg, about 240 μg, about 230 μg, about 220 μg, about 210 μg, about 200 μg, about 190 μg, about 180 μg, about 170 μg, about 160 μg, about 150 μg, about 140 μg, about 130 μg, about 120 μg, about 110 μg, about 100 μg, about 90 μg, about 80 μg, about 70 μg, about 60 μg, about 50 μg, about 40 μg, about 30 μg, or about 20 μg.

Dosage forms for the topical or transdermal administration of compounds of the invention include powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches, and drops. The active ingredient may be mixed under sterile conditions with a pharmaceutically-acceptable carrier, and with any buffers, or propellants which may be required.

The ointments, pastes, creams and gels may contain, in addition to the active ingredient, excipients, such as animal and vegetable fats, oils, waxes, paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc and zinc oxide, or mixtures thereof.

Powders and sprays can contain, in addition to the active ingredient, excipients such as lactose, talc, silicic acid, aluminum hydroxide, calcium silicates and polyamide powder or mixtures of these substances. Sprays can additionally contain customary propellants such as chlorofluorohydrocarbons and volatile unsubstituted hydrocarbons, such as butane and propane.

Transdermal patches have the added advantage of providing controlled delivery of compounds of the invention to the body. Such dosage forms can be made by dissolving, dispersing or otherwise incorporating one or more compounds of the invention in a proper medium, such as an elastomeric matrix material. Absorption enhancers can also be used to increase the flux of the compound across the skin. The rate of such flux can be controlled by either providing a rate-controlling membrane or dispersing the compound in a polymer matrix or gel.

Pharmaceutical compositions of this invention suitable for parenteral administrations comprise one or more compounds of the invention in combination with one or more pharmaceutically-acceptable sterile isotonic aqueous or non-aqueous solutions, dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use, which may contain antioxidants, buffers, solutes which

render the formulation isotonic with the blood of the intended recipient or suspending or thickening agents.

Examples of suitable aqueous and nonaqueous carriers which may be employed in the pharmaceutical compositions of the invention include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

These compositions may also contain adjuvants such as wetting agents, emulsifying agents and dispersing agents. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like in the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as aluminum monosterate and gelatin.

While it is possible for a compound of the present invention to be administered alone, it is preferable to administer the compound as a pharmaceutical formulation (composition). The pharmaceutical compositions of the invention comprise a compound or compounds of the invention as an active ingredient in admixture with one or more pharmaceutically-acceptable carriers and, optionally, with one or more other compounds, drugs or other materials. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the animal. Pharmaceutically-acceptable carriers are well known in the art. Regardless of the route of administration selected, the compounds of the present invention are formulated into pharmaceutically-acceptable dosage forms by conventional methods known to those of skill in the art. See, *e.g., Remington's Pharmaceutical Sciences.*

HSA has been used for fluid resuscitation and, more recently, for chronic liver and renal failure (Rozga J, et al. Human albumin: old, new, and emerging applications. *Ann Transplant* 2013, 18:205-217), and the fraction of 5% HSA under 5,000 Daltons, LMWF of HSA, has been shown to inhibit the release of inflammatory cytokines (Thomas GW, et al. Anti-Inflammatory Activity in the Low Molecular Weight Fraction of Commercial Human Serum Albumin (LMWF5A). *J Immunoassay Immunochem* 2016, 37(1):55-67; Bar-Or D, et al. Commercial human albumin preparations for clinical use are immunosuppressive in vitro. *Crit Care Med* 2006, 34(6):1707-1712). Clinical trials, in which the efficacy and safety of LMWF of HSA as a treatment for

osteoarthritis of the knee were tested, have shown that injection of LMWF of HSA into the knee joint improves function and decreases pain in osteoarthritic knees, implicating an anti-inflammatory mode of action *in vivo* ((Rozga J, et al. Human albumin: old, new, and emerging applications. *Ann Transplant* 2013, 18:205-217). Other anti-inflammatory drugs, such as NSAIDs, have long been used to treat pain and swelling in osteoarthritis (Amoako AO, Pujalte GG: Osteoarthritis in young, active, and athletic individuals. *Clin Med Insights Arthritis Musculoskelet Disord* 2014, 7:27-32). Thus, the inventors concluded that LMWF of HSA may have a mechanism of action that is similar to that of NSAIDs, *i.e.*, blocking the enzymatic function of COX2 and the subsequent downstream production of PGs (Botting RM: Vane's discovery of the mechanism of action of aspirin changed our understanding of its clinical pharmacology. *Pharmacol Rep* 2010, 62(3):518-525). As discussed in Example 1 below, the inventors have found that unexpectedly, LMWF of HSA super-induces COX2 when HSF-OAs are stimulated with either IL-1β or TNFα. They also observed a more pronounced response in IL-1β-stimulated, LMWF of HSA -treated HSF-OAs as compared to TNFα-stimulated, LMWF of HSA -treated cells. Several cytokines and chemokines, including IL-1β and TNFα, have been implicated in the progression of OA; however, important differences exist between IL-1β and TNFα with respect to OA. Increased IL-1β levels are found in OA sera compared to normal sera (Sohn DH, *et al*: Plasma proteins present in osteoarthritic synovial fluid can stimulate cytokine production via Toll-like receptor 4. *Arthritis Res Ther* 2012, 14(1):R7), and synovial membrane and cartilage samples from patients with OA show higher levels of IL-1β-converting enzyme, which is required to process the precursor form of IL-1β into mature cytokine (Saha N, et al. Interleukin-1beta-converting enzyme/caspase-1 in human osteoarthritic tissues: localization and role in the maturation of interleukin-1beta and interleukin-18. *Arthritis Rheum* 1999, 42(8):1577-1587). In a mouse model of arthritis, IL-1 blockade prevents further disease progression, whereas inhibition of TNFα only decreases inflammation within the joint (Joosten LA, et al. IL-1 alpha beta blockade prevents cartilage and bone destruction in murine type II collagen-induced arthritis, whereas TNF-alpha blockade only ameliorates joint inflammation. *J Immunol* 1999, 163(9):5049-5055). TNFα is significantly increased in OA synovial fluid when compared to normal synovial fluid but is absent in OA sera (Sohn DH, *et al*: Plasma proteins present in osteoarthritic synovial fluid can stimulate cytokine production via Toll-like receptor 4. *Arthritis Res Ther* 2012, 14(1):R7). The inventors observed differences in the effects of IL-1β versus TNFα stimulation in the presence of

LMWF of HSA were pronounced with respect to COX2. Induction of COX2 in IL-1β-stimulated, LMWF of HSA-treated cells was much higher compared to TNFα-stimulated, LMWF of HSA-treated cells; however, quantification of PGE2 release from IL-1β-stimulated cells in the presence of LMWF of HSA showed no significant difference. It was only under TNFα-stimulated, LMWF of HSA -treated conditions that a significant increase in PGE2 was observed. It is important to note that the relative levels of PGE2 were 10-fold higher under IL-1β-stimulated conditions.

Considering that COX2 expression and PGE2 production are either the same or increased with LMWF-5A under pro-inflammatory cytokine conditions, one may expect that LMWF of HSA injection into the OA knee would elicit a localized inflammatory response. Eliciting an inflammatory response as a treatment has been coined as prolotherapy, which is characterized by redness, swelling, and pain following injections of prolotherapeutics, such as hypertonic dextrose and morrhuate sodium (Rabago D, Patterson JJ: Prolotherapy: an effective adjunctive therapy for knee osteoarthritis. *J Am Osteopath Assoc* 2013, 113(2):122-123). The premise of prolotherapy is to elicit an inflammatory response that acts to trigger resolution and healing signaling cascades. Based on clinical trial data, LMWF of HSA does not cause a localized inflammatory response *in vivo*, as patients do not experience joint swelling but do experience rapid pain relief that persists 12 weeks post-injection (Bar-Or D, *et al*: A randomized clinical trial to evaluate two doses of an intra-articular injection of LMWF-5A in adults with pain due to osteoarthritis of the knee. *PLoS One* 2014, 9(2):e87910). Additional evidence that LMWF of HSA does not elicit an acute inflammatory response is that PBMCs stimulated with lipopolysaccharide release significantly less TNFα into the medium when co-treated with LMWF of HSA (Thomas GW, et al. Anti-Inflammatory Activity in the Low Molecular Weight Fraction of Commercial Human Serum Albumin (LMWF5A). *J Immunoassay Immunochem* 2016, 37(1):55-67), and treatment of HSF-OAs with LMWF of HSA and either IL-1β or TNFα does not increase IL-1β release into the media.

Because the inventors did not observe hallmarks of an acute inflammatory response either *in vivo* or *in vitro*, LMWF of HSA may circumvent this response and directly initiate healing and regeneration in the knee. Recent evidence has shown that inhibiting PGE2 degradation, thus increasing the tissue PGE2 concentration, potentiates multi-tissue regeneration and increases hematopoiesis and bone marrow stem cell fitness (Zhang Y, *et al*: TISSUE REGENERATION. Inhibition of the prostaglandin-degrading enzyme 15-PGDH potentiates tissue regeneration. *Science* 2015, 348(6240):aaa2340). Fibroblasts found in the synovial fluid are closely related to

bone marrow stem cells (Jones EA, et al. Enumeration and phenotypic characterization of synovial fluid multipotential mesenchymal progenitor cells in inflammatory and degenerative arthritis. *Arthritis Rheum* 2004, 50(3):817-827) and may be a source of resident stem cells within the knee. Previously, the inventors have shown that LMWF of HSA drives chondrocyte condensation in human mesenchymal stem cells (hMSCs) (Bar-Or D, et al. Low Molecular Weight Fraction of Commercial Human Serum Albumin Induces Morphologic and Transcriptional Changes of Bone Marrow-Derived Mesenchymal Stem Cells. *Stem Cells Transl Med* 2015, 4(8):945-955), and inhibition of *COX2* disrupts hMSC chondrogenesis (Pountos I, et al. NSAIDS inhibit in vitro MSC chondrogenesis but not osteogenesis: implications for mechanism of bone formation inhibition in man. *J Cell Mol Med* 2011, 15(3):525-534). Thus, injection of LMWF of HSA may influence multiple cell populations within the knee, synoviocytes, resident stem cells, and chondrocytes, to regenerate damaged cartilage through upregulation of COX2 and PGE2. Furthermore, we observed a significant increase in PGD2 release from LMWF of HSA -treated HSF-OAs under IL-1β and TNFα conditions. Increased PGD2 may trigger an anti-inflammatory/pro-resolution cascade, as it spontaneously undergoes non-enzymatic dehydration and is converted into 15-deoxy-$\Delta^{12,14}$-prostaglandin J2 (15d-PGJ2), a cyclopentenone PG that has been shown to be immuno-modulatory and anti-inflammatory by its ability to inhibit NFκB signaling and cytokine release and to act as an agonist of PPARγ (Buckley CD, et al. Proresolving lipid mediators and mechanisms in the resolution of acute inflammation. *Immunity* 2014, 40(3):315-327). The inventors attempted to measure 15d-PGJ2 using a commercially available competitive ELISA but found that LMWF of HSA interfered with quantification, resulting in artificially increased values. Nonetheless, the data presented herein clearly shows that LMWF of HSA significantly increases anti-inflammatory PGD2 release. Since increased release of PGs may be a key aspect of the therapeutic action of LMWF of HSA and because NSAIDs inhibit the production of all subclasses of PGs due to the inhibition of upstream COX2 enzymatic action, these results have influenced a current clinical trial evaluating LMWF of HSA, resulting in the exclusion of NSAID use by trial participants (NCT02556710).

The following experimental results are provided for purposes of illustration and are not intended to limit the scope of the invention.

EXAMPLES

Example 1:

The ability to decrease inflammation and promote healing is important in the intervention and management of a variety of disease states, including osteoarthritis of the knee (OAK). Even though COX2 has an established pro-inflammatory role, evidence suggests it is also critical to the resolution that occurs after the initial activation phase of the immune response. In this example, the effects of the LMWF of HSA wherein at least one of the components of the LMWF comprises DA-DKP, an agent that has proven to decrease pain and improve function in OAK patients after intra-articular injection, was studied on the expression of COX2 and its downstream products, prostaglandins (PGs).

In the inflammatory environment of OAK, LMWF of HSA (LMWF-5A) treated synoviocytes increase their expression of COX2 and the downstream prostaglandins PGE2 and PGD2. Importantly, no inflammatory responses have been observed in either OAK patients or cell culture upon intra-articular injection or treatment with LMWF-5A. LMWF-5A is believed to increase COX2 and downstream PGE2 release from synoviocytes into the synovial fluid, inducing regeneration of cartilage, as they have been implicated in promoting chondrogenesis and tissue regeneration. Furthermore, the inventors have previously shown that LMWF-5A increases chondrocyte condensation in stem cells. Additionally, increased PGD2 release from synoviocytes observed upon LMWF-5A treatment may trigger resolution of inflammation and healing via NF-κB inhibition and PPARγ activation.

Fibroblast-like synoviocytes from the synovial membrane of OAK patients were treated with LMWF of HSA or saline as a control with or without the addition of cytokine (interleukin-1β [IL-1β] or tumor necrosis factor α [TNFα]) to elicit an inflammatory response. Cells were harvested for RNA and protein at 2, 4, 8, 12, and 24 h, and media was collected at 24 h for analysis of secreted products. *COX2* mRNA expression was determined by qPCR, and COX2 protein expression was determined by western blot analysis. Levels of prostaglandin E2 (PGE2) and prostaglandin D2 (PGD2) in the media were quantified by competitive ELISA. In the presence of cytokine, LMWF of HSA increased the expression of both *COX2* mRNA and protein, and this increase was significant compared to that observed with cytokine alone. Downstream of COX2, the levels of PGE2 were increased only in TNFα-stimulated cells; however, in both IL-1β- and TNFα-stimulated cells, LMWF of HSA increased the release of the anti-inflammatory PGD2.

Thus, the LMWF of HSA appears to trigger increased anti-inflammatory PG signaling, and this may be a primary component of its therapeutic mode of action in the treatment of OAK.

Methods for Example 1:

The LMWF of HSA was produced at Ampio Pharmaceuticals (Englewood, CO) as previously described by Bar-Or (Bar-Or, D. *et al*: A randomized clinical trial to evaluate two doses of an intra-articular injection of LMWF-5A in adults with pain due to osteoarthritis of the knee. *PLoS One* 2014, 9(2):e87910). Briefly, 5% HSA (Octapharma, Hoboken, NJ) was subjected to tangential flow filtration through a PVDF membrane with a 5 kDa molecular weight cutoff. The <5 kDa fraction was aseptically filled into glass vials, sealed, and stored in the dark at room temperature.

Cell culture

Human synovial fibroblasts from patients with osteoarthritis (HSF-OA; Asterand, Detroit, MI) were maintained in Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM/F12; ThermoFisher Scientific, Waltham, MA) containing 20% fetal bovine serum (FBS). Before plating for experiments, the cells were fed every two to three days with media containing 10% FBS for two media changes.

For the time course of COX2 mRNA and protein expression, HSF-OAs ($1x10^5$ cells) were plated in each well of a 24-well plate in 500 µL DMEM/F12 containing 10% FBS and incubated at 37°C and 5% CO_2. 500 µL saline or LMWF-5A +/- IL-1β (R&D Systems, Minneapolis, MN) or TNFα (ThermoFisher Scientific, Waltham, MA) (final concentration of 10 ng/mL) were added, and the cells were treated for 2, 4, 8, 12, or 24 h before harvesting for RNA or protein. For the evaluation of prostaglandin release, HSF-OAs were plated and treated as above. After 24 h, the media was collected, treated if necessary (as described below), and stored frozen until use.

Quantitative real-time PCR (qPCR) for COX2

RNA was isolated from the treated HSF-OAs using the miRNeasy kit (Qiagen, Valencia, CA), with 1 min of vortexing for homogenization. 0.5 µg of total RNA was then reverse transcribed into cDNA with the Qiagen QuantiTect kit. qPCR was then performed in duplicate using SYBR Green I Master Mix (Roche Diagnostics, Indianapolis, IN), a RT^2 qPCR primer assay for *COX2* (Qiagen), and a QuantiTect primer assay for 18S rRNA (Qiagen) on a Roche 480 Lightcycler. Relative gene expression was calculated using the comparative threshold cycle ($\Delta\Delta C_T$) method versus a 0 h untreated control, with normalization to 18S rRNA expression.

COX2 western blot analysis

HSF-OAs, as plated above, were lysed in 50 µL lysis buffer (Qproteome Mammalian Protein kit; Qiagen) according to manufacturer's instructions and centrifuged at 12,000 x g at 4°C for 10 min to remove the cellular debris. Lysates were prepared for western blot analysis by boiling in Bolt Reducing Buffer and Bolt LDS Sample Buffer (ThermoFisher Scientific, Waltham, MA). The lysates were separated by SDS-PAGE (8%) and subjected to western blot analysis using an anti-COX2 rabbit monoclonal primary antibody (1:1,000, ab62331; Abcam, Cambridge, MA) and a goat anti-rabbit IgG secondary antibody (1:10,000, Cat# 7074P2, Cell Signaling, Danvers, MA). The COX2 protein levels were normalized to α-tubulin after stripping and reprobing with Reblot Plus (Millipore, Billerica, MA) and a horseradish peroxidase-conjugated α-tubulin antibody (1:5,000, DM1A, Cat# 12351S, Cell Signaling, Danvers, MA), respectively.

Prostaglandin enzyme-linked immunosorbent assays (ELISAs)

The levels of PGE2 in the media were analyzed using the Abcam Prostaglandin E2 ELISA kit (Cambridge, MA) following the manufacturer's protocol. The levels of PGD2 in the media were analyzed using the Cayman Chemical Prostaglandin D2-MOX EIA kit (Ann Arbor, MI) following the manufacturer's instructions. Notably, with this kit, the PGD2 in the sample is stabilized upon a 30-min incubation at 60°C with a methyloximating reagent immediately following sample collection.

Statistical analysis

All graphs and figures represent four independent experiments. Graphs represent the mean ± standard error of the mean (SEM), and p-values were calculated using a standard student's paired two-tailed t-test.

Results:

LMWF of HSA increases COX2 in cytokine-stimulated OA synoviocytes

LMWF of HSA inhibits cytokine release *in vitro* (Thomas GW, et al., Anti-Inflammatory Activity in the Low Molecular Weight Fraction of Commercial Human Serum Albumin (LMWF5A). *J Immunoassay Immunochem* 2016, 37(1):55-67; Bar-Or D., et al., Commercial human albumin preparations for clinical use are immunosuppressive in vitro. *Crit Care Med* 2006, 34(6):1707-1712), and clinical trial results support an anti-inflammatory mode of action *in vivo* (Bar-Or D, *et al.*, A randomized clinical trial to evaluate two doses of an intra-articular injection of LMWF-5A in adults with pain due to osteoarthritis of the knee. *PLoS One* 2014, 9(2):e87910).

The mode of action of NSAIDs is to inhibit COX2 function and subsequent PG production (Botting RM. Vane's discovery of the mechanism of action of aspirin changed our understanding of its clinical pharmacology. *Pharmacol Rep* 2010, 62(3):518-525), thus, LMWF of HSA may function by a similar mechanism. Using primary synoviocytes isolated from the knee synovial membrane of patients with OA, *COX2* mRNA was quantified by qPCR and COX2 protein by western blotting over a 24 h time course. HSF-OAs treated with saline and then stimulated with IL-1β or TNFα showed an induction in *COX2* mRNA of 19 to 327-fold when normalized back to untreated cells over a 24 h time period (Figure 1A and B). Surprisingly, over the same time course, HSF-OAs stimulated with IL-1β or TNFα induced *COX2* mRNA expression 52 to 692-fold, an additional ~1.2 to ~4.0-fold higher, in the presence of LMWF of HSA (Figure 1A and B; $p<0.05$ for LMWF-5A + IL-1β at 8, 12, and 24 h and LMWF-5A + TNFα at 2, 4, 8, 12, and 24 h). HSF-OAs incubated with LMWF of HSA without cytokine also showed an increase in *COX2* mRNA of 3 to 13-fold when normalized back to untreated controls over a 24 h time course (Figure 1C; $p<0.05$ for LMWF-5A at 2, 4, and 8 h). Interestingly, when COX2 protein was measured by western blot, significant fold increases in COX2 protein were only observed when OA synoviocytes were stimulated with cytokine in the presence of LMWF of HSA (Figure 2). IL-1β-stimulated, LMWF of HSA-treated cells displayed significantly higher levels of COX2 protein between 8-24 hours (Figure 2A; $p<0.05$ for LMWF-5A + IL-1β at 8, 12, and 24 h). When stimulated with TNFα in the presence of LMWF of HSA, COX2 protein was significantly increased over TNFα-stimulated, saline-treated HSF-OAs at 12 h and approached significance ($p<0.07$) at 24 h post-cytokine exposure (Figure 2B; $p<0.05$ for LMWF-5A + TNFα at 12 h). In contrast to the observed *COX2* mRNA dynamics, there was no difference in COX2 protein without cytokine exposure in OA synoviocytes treated with either LMWF of HSA or saline (Figure 2C). Thus, in OA synoviocytes, both *COX2* mRNA and COX2 protein significantly increase only when these cells are stimulated with IL-1β or TNFα in the presence of LMWF of HSA.

LMWF-of HSA increases prostaglandin release from cytokine-stimulated OA synoviocytes

Considering that COX2 expression is increased under inflammatory conditions in the presence of LMWF of HSA, downstream products of COX2 in this system were studied. Two products, PGE2 and PGD2 were focused on. PGE2 has been implicated in the initial phase of the innate immune response, the clearance of the insult, as well as in the promotion of tissue regeneration (Zhang Y, *et al*: TISSUE REGENERATION. Inhibition of the prostaglandin-

degrading enzyme 15-PGDH potentiates tissue regeneration. *Science* 2015, 348(6240):aaa2340). PGD2 has been linked to the second phase, resolution and healing (Gilroy DW, et al. Inducible cyclooxygenase may have anti-inflammatory properties. *Nat Med* 1999, 5(6):698-701). As described above, OA synoviocytes were treated with saline as a control or LMWF of HSA with or without IL-1β or TNFα. The amount of each prostaglandin secreted into the media was determined with a specific competitive ELISA after 24 h of treatment (Figure 3).

The level of PGE2 in the media of cells treated solely with saline or LMWF of HSA was below the limit of detection (LOD) of this assay (39.1 pg/mL); however, when stimulated with cytokine, OA synoviocytes produced detectable levels of PGE2. Upon treatment with IL-1β or TNFα for 24 h under saline conditions, the media contained 231,000 pg/mL and 26,300 pg/mL of PGE2, respectively (Figure 3A). When the cells were stimulated with IL-1β in the presence of LMWF of HSA, the concentration of PGE2 in the media (251,000 pg/mL) was not significantly different from that found in the media of IL-1β-stimulated cells in the presence of saline (Figure 3A). In contrast, the level of PGE2 in the media of TNF-stimulated, LMWF of HSA-treated cells was increased by 37% (41,500 pg/mL, $p<0.05$) compared to that found in the media of TNF-stimulated, saline-treated cells (Figure 3A).

Similar to PGE2, unstimulated OA synoviocytes exhibited no detectable release of PGD2 (LOD = 2 pg/mL). Upon addition of IL-1β or TNFα to saline-treated cells, the concentration of PGD2 in the media increased to 189 pg/mL and 26.1 pg/mL, respectively (Figure 3B). Interestingly, under these conditions, LMWF of HSA significantly increased the release of PGD2. When compared to cytokine-stimulated controls, PGD2 was 261 pg/mL with IL-1β stimulation and 52.4 pg/ml with TNFα stimulation, signifying 28% and 59% increases, respectively, in the presence of LMWF of HSA ($p<0.05$, Figure 3B). It is important to note that PGE2 is a stable molecule, while PGD2 is unstable and must be chemically modified to prevent its degradation. Thus, in this experiment, the level of PGE2 reflects the accumulation of PGE2 over the 24 h time course, and the level of PGD2 represents a snapshot of the PGD2 release at the time of sample collection.

Example 2

This example demonstrates that LMWF of HSA wherein at least one of the components of the LMWF comprises DA-DKP exhibits a unique immune modulation pattern, disparate from both steroid or NSAID treatment. The enhancement of prostanoid release, specifically 15d-PGD_2, taken

together with a drop in cytokine levels, may favor resolution.

The immune response is a carefully orchestrated series of events designed to counteract the initial insult then direct the clearance of debris and promote healing. Traumatic injury activates the innate immune system through the release of damage-associated molecular patterns or alarmins from injured tissues. Dysregulation can lead to systemic inflammatory response syndrome, multiple organ failure, and chronic inflammation. These patients frequently fall victim to "second hit" opportunistic infections as the result of a compensatory anti-inflammatory response. A better understanding of the innate immune response could help manage complications while allowing for proper immune progression. In this example, the ability of several classes of anti-inflammatory drugs to affect LPS induced cytokine and prostaglandin release from peripheral blood mononuclear cells (PBMC) was evaluated *in vitro*.

Methods for Example 2:

PBMC were cultured in the presence of anti-inflammatory compounds for one hour then stimulated with LPS. TNFα, PGE_2, and 15d-PGD_2 release was then determined by ELISA after 24 hours.

Results:

Three distinct immunomodulation patterns emerged following LPS stimulation of PBMC in the presence of various anti-inflammatories. Dexamethasone, a strong immunosuppressive steroid reduced both cytokine and prostanoid release. With the NSAID, ibuprofen, an almost complete attenuation of prostanglandin release was observed while cytokine levels remained unchanged. The LMWF of HSA exhibited an ability to reduce TNFα release while enhancing the amount PGE_2 and 15d-PGD_2 detected. Incubating LMFW of HSA together with ibuprofen negated the observed prostanoid enhancement without effecting the suppression of TNFα. See Figure 5.

Example 3

The data in this example demonstrate that LMWF of HSA wherein at least one of the components of the LMWF comprises DA-DKP inhibits NFκB signaling on a global level through regulation of NFκB relevant transcripts and miRNA. Systemic administration of LMWF of HSA may thus ameliorate inflammation in trauma patients.

A major pathway stimulated by trauma-induced inflammation is the NFκB signaling network. NFκB signaling results in downstream cellular responses that include production of pro-inflammatory cytokines, such as IL-1β and TNFα. Systemic inflammation may promote

multiple-organ failure during severe trauma, in which NFκB signaling plays a central role. Historically, severe trauma patients have been treated with HSA to decrease tissue edema and for fluid resuscitation.

Methods:

Human embryonic kidney cells (HEK-293T) expressing a luciferase reporter gene driven by four NFκB-response elements were treated with either saline control or LMWF of HSA in the presence of IL-1β or TNFα. Luciferase activity was measured 3h following cytokine exposure and normalized for cell viability. Human synovial primary fibroblasts (HSF-OA) were also used. To determine differential gene expression, RNA sequencing of whole transcriptome and miRNA expression was performed on HSF-OA either treated with saline or LMWF of HSA for 24h with or without IL-1β stimulation. Significantly differentially expressed transcripts were identified in saline versus saline+IL-1β (SvS+I) and LMWF of HSA versus LMWF of HSA+IL-1β (LvL+I). Ingenuity® Pathway Analysis (IPA) was used to determine relevant gene networks differentially regulated by LMWF of HSA versus saline in IL-1β-stimulated cells.

Results:

In TNFα-stimulated HEK-293T cells, NFκB transcriptional activity was decreased by ~30% in LMWF of HSA treated cells. A known transcriptional target gene of NFκB, Interleukin-8 (IL-8) was differentially induced when comparing SvS+I and LvL+I gene lists, indicating a ~700-fold decrease in IL-8 mRNA induction in the presence of LMWF of HSA. Differential expression of several mediators of NFκB signaling were also observed, including NFκB inhibiting kinase (NIK), NFκB2, and RELB, all members of the non-canonical NFκB pathway. All of these transcripts decreased or did not increase in the presence of LMWF of HSA versus saline when cells were stimulated with IL-1β. Furthermore, treatment with LMWF of HSA completely blocked expression of miR-486. By repressing negative NFκB feedback loops, miR-486 perpetuates NFκB signaling, lending more support to LMWF of HSA inhibition of NFκB signaling.

All of the documents cited herein are incorporated herein by reference.

While various embodiments of the present invention have been described in detail, it is apparent that modifications and adaptations of those embodiments will occur to those skilled in the art. It is to be expressly understood, however, that such modifications and adaptations are within the scope of the present invention, as set forth in the following exemplary claims.

WHAT IS CLAIMED IS:

1. A method of inhibiting inflammation, the method comprising administering to an individual in need thereof an effective amount of a pharmaceutical composition comprising a low molecular weight fraction (LMWF) of human serum albumin (HSA), wherein the individual is not administered a cyclooxygenase-2 (COX-2) antagonist within the period of time ranging from about one hour before to about 6 hours after administration of the pharmaceutical composition.

2. The method of claim 1, wherein the LMWF of HSA contains components having a molecular weight of less than 5000.

3. The method of claim 1, wherein the LMWF of HSA contains components having a molecular weight less than 3000.

4. The method of claim 1, wherein at least one of the components in the LMWF of HSA comprises DA-DKP.

5. The method of claim 1, wherein the LMWF of HSA comprises one or more compounds selected from the group consisting of N-acetyl tryptophan and caprylic acid.

6. The method of claim 1, wherein the individual receiving the pharmaceutical composition is not administered a COX-2 antagonist within a time period selected from the group consisting of at least about one hour, at least about 12 hours, at least about one day, at least about two days, at least about three days, at least about four days, at least about five days, at least about six days, at least about seven days, at least about eight days or at least about nine days and 10 days, prior to administration of the pharmaceutical composition.

7. The method of claim 1, wherein the individual receiving the pharmaceutical composition is not administered a COX-2 antagonist during the period of time in which at least one active ingredient in the pharmaceutical composition exerts its effect.

8. The method of claim 1, wherein the individual receiving the pharmaceutical composition is not administered a COX-2 antagonist within a time period selected from the group consisting of at least 6 hours, at least 12 hours, at least about 24 hours, at least about two days, at least about three days, at least about four days, at least about five days, at least about six days, at last about one week, at least about two weeks, at least about three weeks, at least about one month, at least about two months, at least about three months, at least about four months at least about five months or at least about six months after administration of a pharmaceutical composition of the invention.

9. The method of claim 7, wherein the composition that reduces COX-2 activity comprises a chemical selected from the group consisting of acetylsalicylic acid (aspirin), 2-(4-isobutylphenyl)propanoic acid (ibuprofen), *N*-(4-hydroxyphenyl)ethanamide (paracetamol), (S)-6-methoxy-α- methyl-2-naphthaleneacetic acid (naproxen), 2-[(2,6-dichlorophenyl)amino] benzeneacetic acid (diclofenac), 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl] benzenesulfonamide (celecoxib), 4-[4-(methylsulfonyl)phenyl]-3-phenyl-2(5H)-furanone (rofecoxib), and 4-(5-Methyl-3-phenylisoxazol-4-yl)benzolsulfonamid (valdecoxib).

10. A method of treating a T-cell mediated disease, the method comprising administering to an individual in need thereof an effective amount of a pharmaceutical composition comprising a low molecular weight fraction (LMWF) of human serum albumin (HSA), wherein the individual is not administered a cyclooxygenase-2 (COX-2) antagonist within the period of time ranging from about one hour before to about six hours after administration of the pharmaceutical composition.

11. The method of claim 10, wherein the T-cell mediated disease is graft rejection, graft versus host disease, an unwanted delayed-type hypersensitivity reaction, a T-cell mediated pulmonary disease, an autoimmune disease or an inflammatory disease.

12. The method of claim 10, wherein the T-cell-mediated disease is selected form the group consisting of multiple sclerosis, neuritis, polymyositis, psoriasis, vitiligo, Sjogren's syndrome, rheumatoid arthritis, Type 1 diabetes, autoimmune pancreatitis, inflammatory bowel diseases, Crohn's disease, ulcerative colitis, celiac disease, glomerulonephritis, scleroderma, sarcoidosis, autoimmune thyroid diseases, Hashimoto's thyroiditis, Graves disease, myasthenia gravis, Addison's disease, autoimmune uveoretinitis, pemphigus vulgaris, primary biliary cirrhosis, pernicious anemia and systemic lupus erythematosis.

13. The method of claim 10, wherein the T-cell-mediated disease is pulmonary fibrosis or idiopathic pulmonary fibrosis.

14. The method of claim 10, wherein the T-cell-mediated disease is an inflammatory disease.

15. The method of claim 10, wherein the LMWF of HSA contains components having a molecular weight of less than 5000.

16. The method of claim 10, wherein the LMWF of HSA contains components having a molecular weight less than 3000.

17. The method of claim 10, wherein at least one of the components in the LMWF of HSA comprises DA-DKP.

18. The method of claim 10, wherein the LMWF of HSA comprises one or more compounds selected from the group consisting of N-acetyl tryptophan and caprylic acid.

19. The method of claim 10, wherein the individual receiving the pharmaceutical composition is not administered a COX-2 antagonist within a time period selected from the group consisting of at least about one hour, at least about 12 hours, at least about one day, at least about two days, at least about three days, at least about four days, at least about five days, at least about six days, at least about seven days, at least about eight days or at least about nine days and 10 days, prior to administration of the pharmaceutical composition.

20. The method of claim 10, wherein the individual receiving the pharmaceutical composition is not administered a COX-2 antagonist during the period of time in which at least one active ingredient in the pharmaceutical composition exerts its effect.

21. The method of claim 10, wherein the individual receiving the pharmaceutical composition is not administered a COX-2 antagonist within a time period selected from the group consisting of at least 6 hours, at least 12 hours, at least about 24 hours, at least about two days, at least about three days, at least about four days, at least about five days, at least about six days, at last about one week, at least about two weeks, at least about three weeks, at least about one month, at least about two months, at least about three months, at least about four months at least about five months or at least about six months after administration of a pharmaceutical composition of the invention.

22. The method of claim 20, wherein the composition that reduces COX-2 activity comprises a chemical selected from the group consisting of acetylsalicylic acid (aspirin), 2-(4-isobutylphenyl)propanoic acid (ibuprofen), *N*-(4-hydroxyphenyl)ethanamide (paracetamol), (S)-6-methoxy-α- methyl-2-naphthaleneacetic acid (naproxen), 2-[(2,6-dichlorophenyl)amino] benzeneacetic acid (diclofenac), 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl] benzenesulfonamide (celecoxib), 4-[4-(methylsulfonyl)phenyl]-3-phenyl-2(5H)-furanone (rofecoxib), and 4-(5-Methyl-3-phenylisoxazol-4-yl)benzolsulfonamid (valdecoxib).

23. A method of treating an individual for a joint disease or condition, the method comprising administering to an individual in need thereof an effective amount of a pharmaceutical composition comprising a LMWF of HSA, wherein the individual is not administered a COX-2

antagonist within the period of time ranging from about one hour before to about six hours after administration of the pharmaceutical composition.

24. The method of claim 23, wherein the joint disease or condition is a degenerative joint disease.

25. The method of claim 23, wherein the LMWF of HSA contains components having a molecular weight of less than 5000.

26. The method of claim 23, wherein the LMWF of HSA contains components having a molecular weight less than 3000.

27. The method of claim 23, wherein at least one of the components in the LMWF of HSA comprises DA-DKP.

28. The method of claim 23, wherein the LMWF of HSA comprises one or more compounds selected from the group consisting of N-acetyl tryptophan and caprylic acid.

29. The method of claim 23, wherein the individual receiving the pharmaceutical composition is not administered a COX-2 antagonist within a time period selected from the group consisting of at least about one hour, at least about 12 hours, at least about one day, at least about two days, at least about three days, at least about four days, at least about five days, at least about six days, at least about seven days, at least about eight days or at least about nine days and 10 days, prior to administration of the pharmaceutical composition.

30. The method of claim 23, wherein the individual receiving the pharmaceutical composition is not administered a COX-2 antagonist during the period of time in which at least one active ingredient in the pharmaceutical composition exerts its effect.

31. The method of claim 23, wherein the individual receiving the pharmaceutical composition is not administered a COX-2 antagonist within a time period selected from the group consisting of at least 6 hours, at least 12 hours, at least about 24 hours, at least about two days, at least about three days, at least about four days, at least about five days, at least about six days, at last about one week, at least about two weeks, at least about three weeks, at least about one month, at least about two months, at least about three months, at least about four months at least about five months or at least about six months after administration of a pharmaceutical composition of the invention.

32. The method of claim 30, wherein the composition that reduces COX-2 activity comprises a chemical selected from the group consisting of acetylsalicylic acid (aspirin), 2-(4-

isobutylphenyl)propanoic acid (ibuprofen), *N*-(4-hydroxyphenyl)ethanamide (paracetamol), (S)-6-methoxy-α- methyl-2-naphthaleneacetic acid (naproxen), 2-[(2,6-dichlorophenyl)amino] benzeneacetic acid (diclofenac), 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl] benzenesulfonamide (celecoxib), 4-[4-(methylsulfonyl)phenyl]-3-phenyl-2(5H)-furanone (rofecoxib), and 4-(5-Methyl-3-phenylisoxazol-4-yl)benzolsulfonamid (valdecoxib).

33. The method of reducing the level of IL-8 in an individual, the method comprising administering to an individual in need thereof an effective amount of a pharmaceutical composition comprising a LMWF of HSA, wherein the individual is not administered a COX-2 antagonist within the period of time ranging from about one hour before to about six hours after administration of the pharmaceutical composition.

Fig. 1A

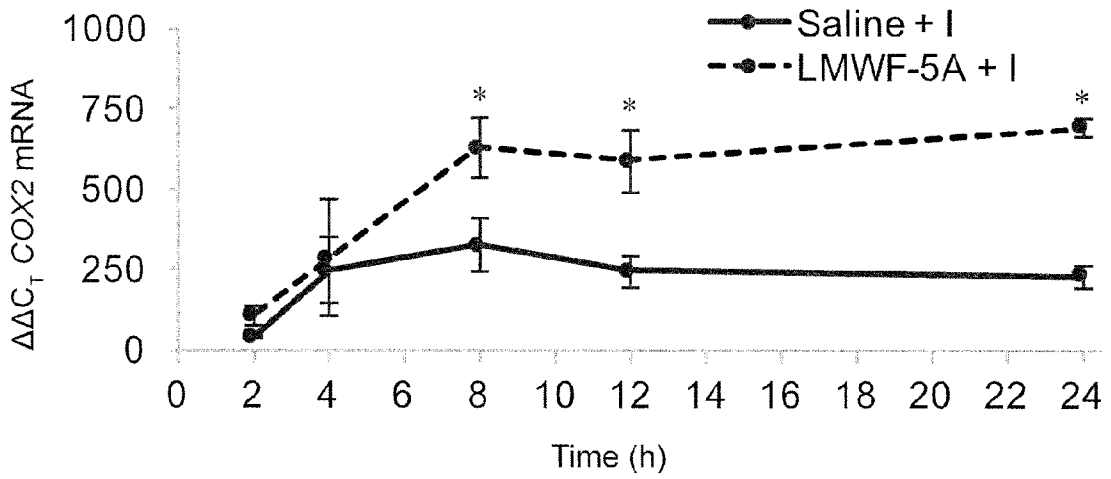


Fig. 1B

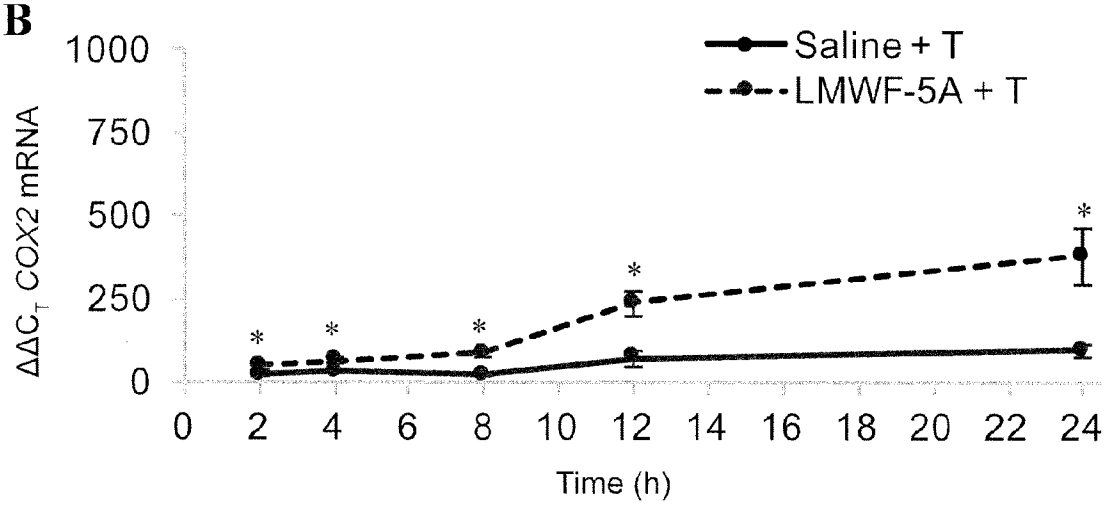


Fig. 1C

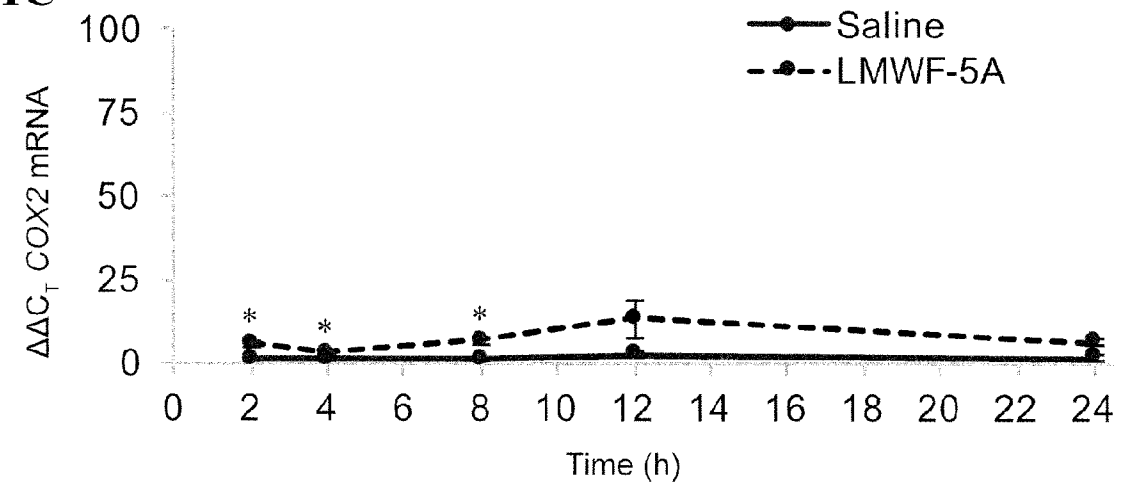


Fig. 2 A

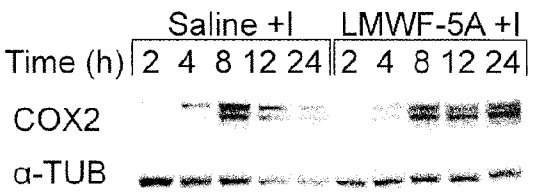


Fig. 2B

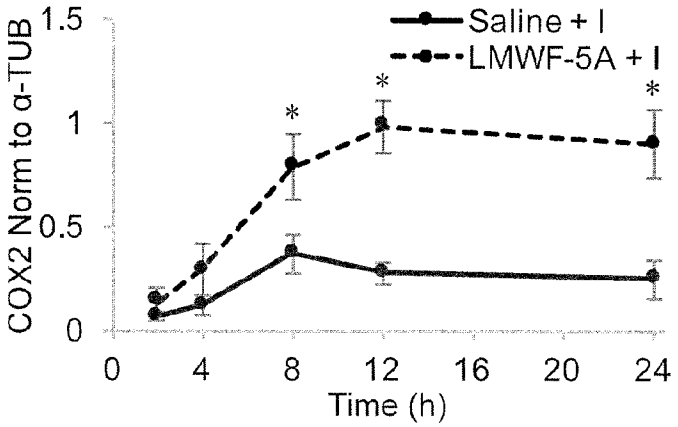


Fig. 2C

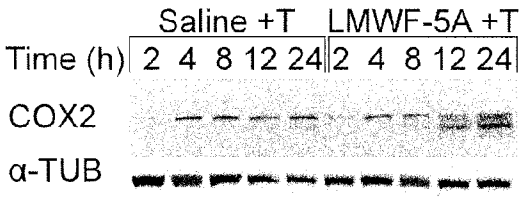


Fig. 2D

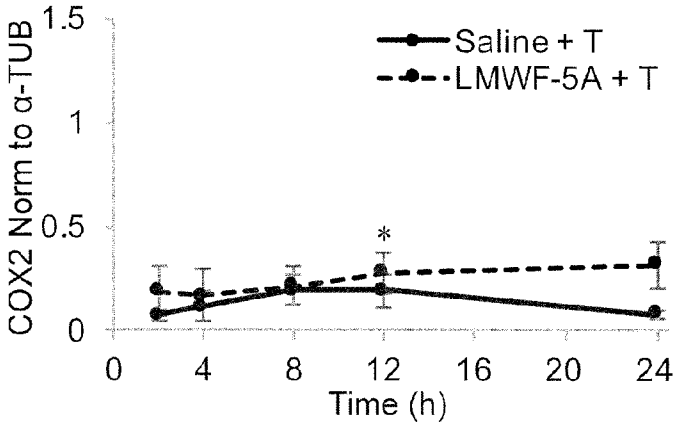


Fig. 2E

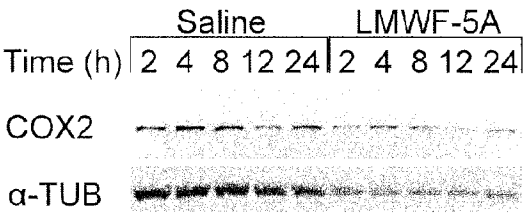


Fig. 2F

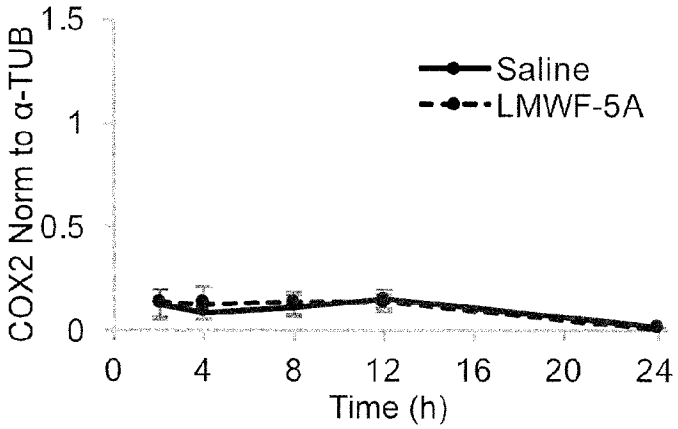
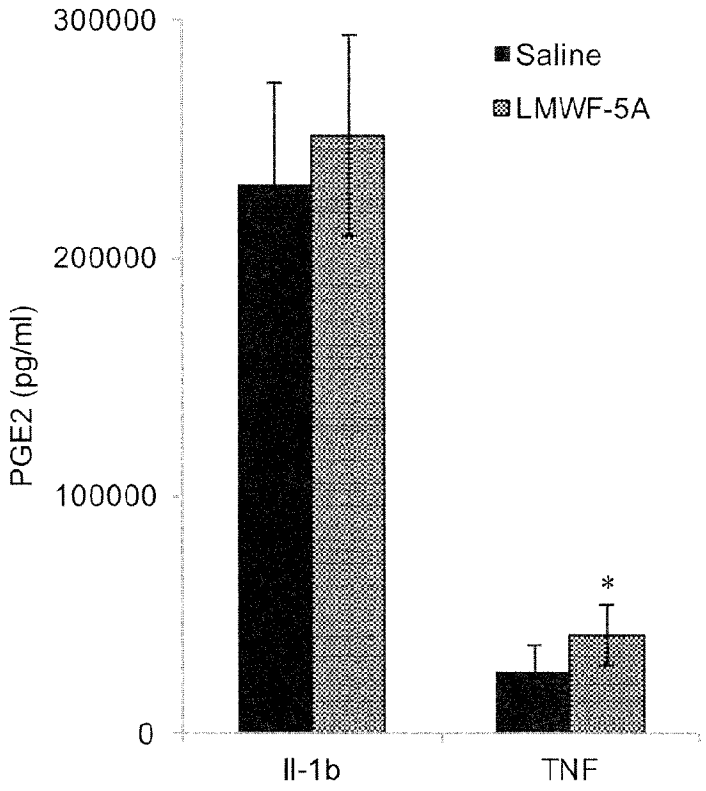


Fig. 3A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200000
100000
0
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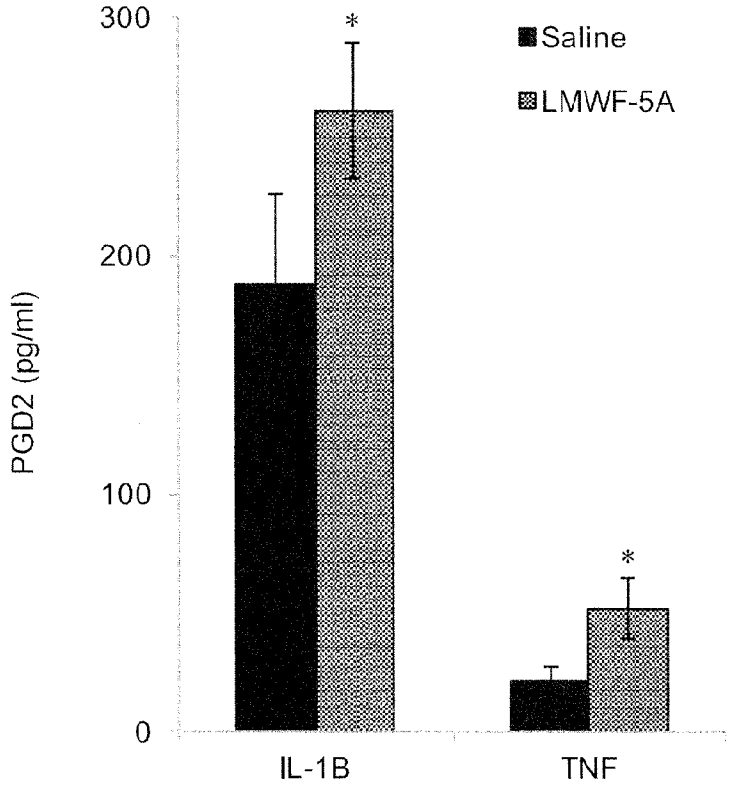
LMWF-5A
*
Il-1b
TNF

Fig. 3B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200
100
0
PGD2 (pg/ml)
Saline
LMWF-5A
*
*
IL-1B
TNF

Fig. 4

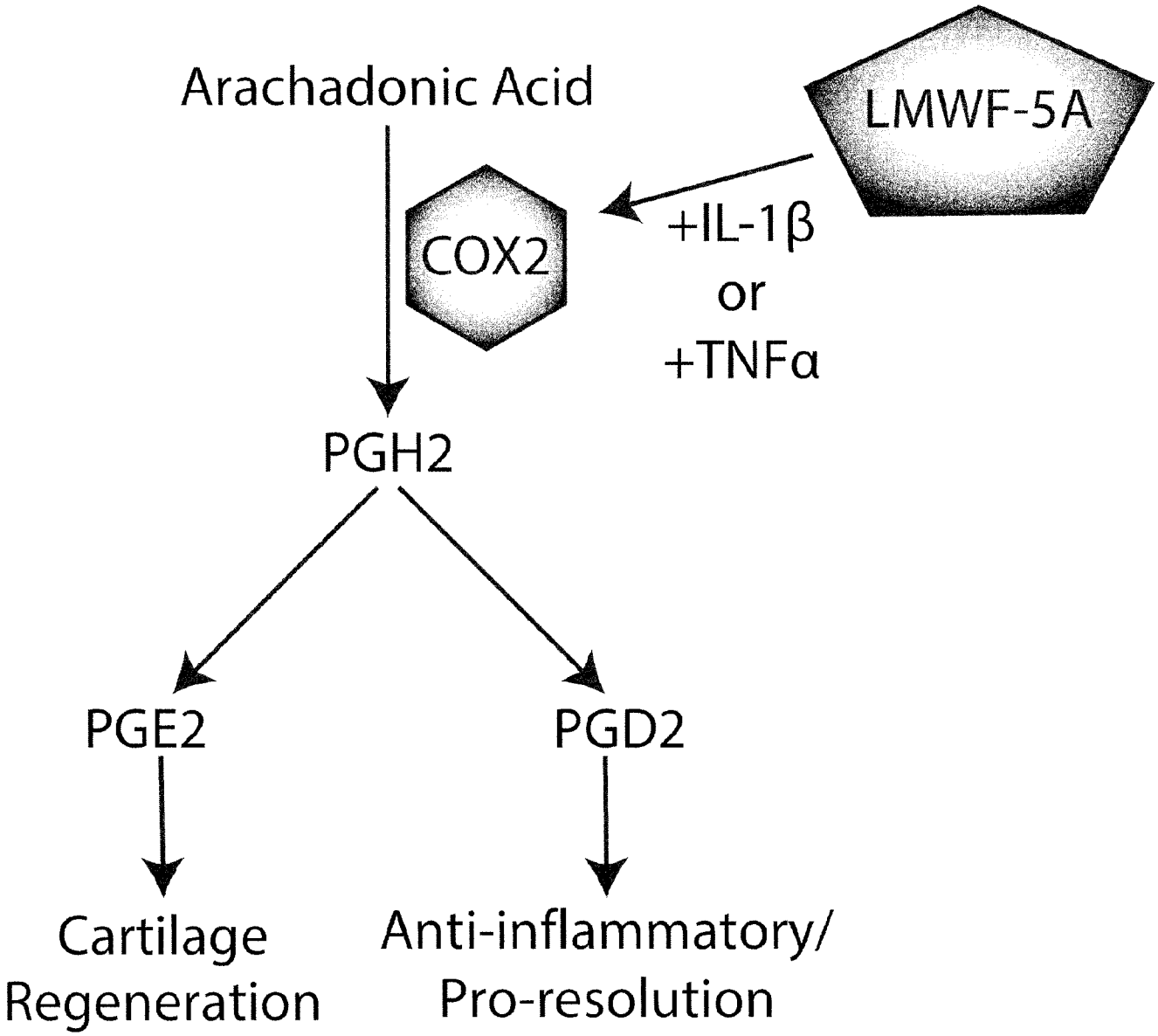
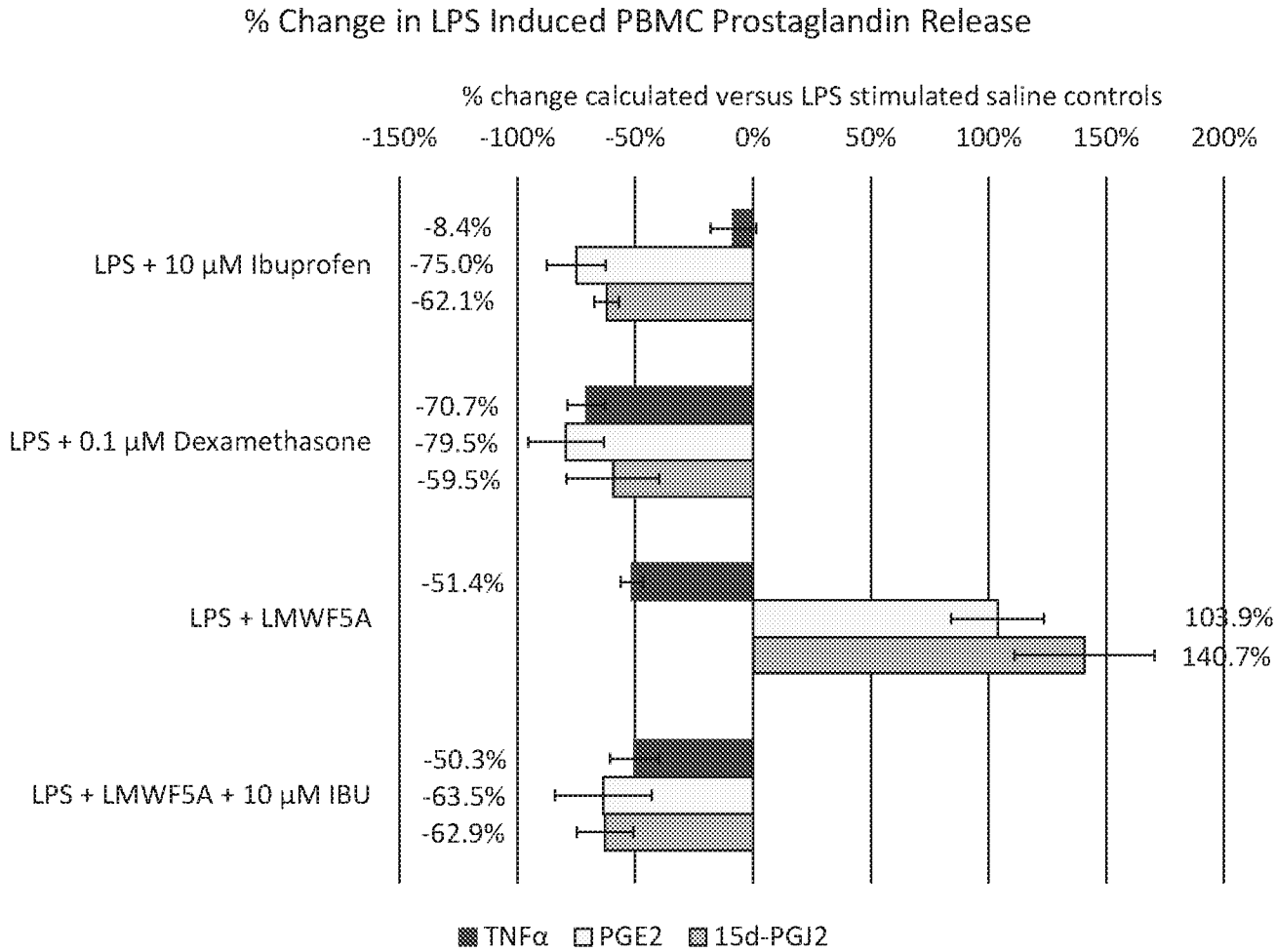

Arachadonic Acid
LMWF-5A
COX2
+IL-1β
or
+TNFα
PGH2
PGE2
PGD2
Cartilage
Regeneration
Anti-inflammatory/
Pro-resolution

FIG. 5


% Change in LPS Induced PBMC Prostaglandin Release
% change calculated versus LPS stimulated saline controls
-150%
-100%
-50%
0%
50%
100%
150%
200%
LPS + 10 µM Ibuprofen
-8.4%
-75.0%
-62.1%
LPS + 0.1 µM Dexamethasone
-70.7%
-79.5%
-59.5%
LPS + LMWF5A
-51.4%
103.9%
140.7%
LPS + LMWF5A + 10 µM IBU
-50.3%
-63.5%
-62.9%
TNFα
PGE2
15d-PGJ2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US16/38774

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 38/12 (2016.01)
CPC - A61K 38/12, 31/167, 9/0019, 45/06
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8): A61K 38/12 (2016.01)
CPC: A61K 38/12, 31/167, 9/0019, 45/06

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatSeer (US, EP, WO, JP, DE, GB, CN, FR, KR, ES, AU, IN, CA, INPADOC Data); EBSCO; PubMed; Google/Google Scholar; diketopiperazine, DKP, inflammatory, T-cell, cytokine, LMWF, HSA, serum, albumin, COX-2, caprylic, aspirin, ibuprofen, joint, IL-8, interleukin

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2014/0294738 A1 (AMPIO PHARMACEUTICALS, INC.) 02 October 2014; abstract; paragraphs [0003], [0007]-[0008]	1-9
Y	WO 2015/028657 A1 (TAKEDA GMBH) 05 March 2015; page 37, lines 19-27; page 38, lines 1-12; page 133, lines 16-25	1-33
Y	US 2014/0256642 A1 (AMPIO PHARMACEUTICALS, INC.) 11 September 2014; abstract; paragraphs [0005]-[0009]	10-33
Y	WO 2014/121210 A1 (AMPIO PHARMACEUTICALS, INC.) 07 August 2014; claims 1, 14	5
Y	US 2010/0240602 A1 (BURKE, TG et al.) 23 September 2010; paragraphs [0021], [0040], [0074]	9, 22, 32
Y	US 8,969,308 B2 (AMPIO PHARMACEUTICALS, INC.) 03 March 2015; abstract; column 2, lines 47-52; column 4, lines 16-23, 60-66; column 5, lines 1-11; column 13, lines 8-31; column 16, lines 9-24; column 17, lines 15-23	10-22
Y	US 2015/0051223 A1 (AMPIO PHARMACEUTICALS, INC.) 19 February 2015; abstract; paragraphs [0007]-[0008], [0018]	33

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 August 2016 (25.08.2016)	14 SEP 2016
Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)