1. REVIEW OF LITERATURE

1.1 Inflammation

Inflammation is an important pathologic process; it has been recognized for centuries. Almost 2,000 years ago, the Roman physician Celsus recognized the warmth, redness, swelling, and pain associated with what later became known as "inflammation."[1] The warmth, redness, swelling, and pain Celsus reported is caused by a series of cellular and tissue responses to some injurious agent. The responses are directed at destroying the inciting agent or, at least, rendering it harmless. In the process, inflammation also prevents the spread of the inciting injurious agent. All this activity may cause damage or destruction to normal tissue in the immediate area; the inflammatory process cleans up resulting debris and starts restoring tissues to their normal states.

1.11 Significance of inflammation

Inflammation is a fundamental and common pathologic process. When recognized by practitioners, it indicates that the body is struggling to deal with some invading agent or damaged tissues. It is a process seen in many disease states from splinters to syphilis, from arthritis to AIDS. There are a number of oral diseases in which inflammation is a significant component: periodontal disease, pulpal disease, periapical disease, and oral infections are but a few examples.

1.12 Etiology of inflammation

Many agents can provoke an inflammatory response. The painful redness that follows over-exposure to the sun is one common example of a *physical agent*, solar radiation produces inflammation.[2] Chemicals are another group of agents that can cause inflammation. As but one example of the inflammation-causing effects of some chemicals, turpentine, a common paint solvent, was used by scientists to provoke inflammation in laboratory animals. Further, inflammation is a component of most hypersensitivity reactions. The pain and redness so commonly associated with rheumatoid arthritis attest to the relationship of inflammation and hypersensitivity. Finally, and most obviously, biologic agents cause inflammation. The pain, swelling, and pus associated with a severe bacterial infection are cardinal signs of inflammation. From the examples presented above, it is clear that any substance that can elicit an immune response will elicit an inflammatory response as well.

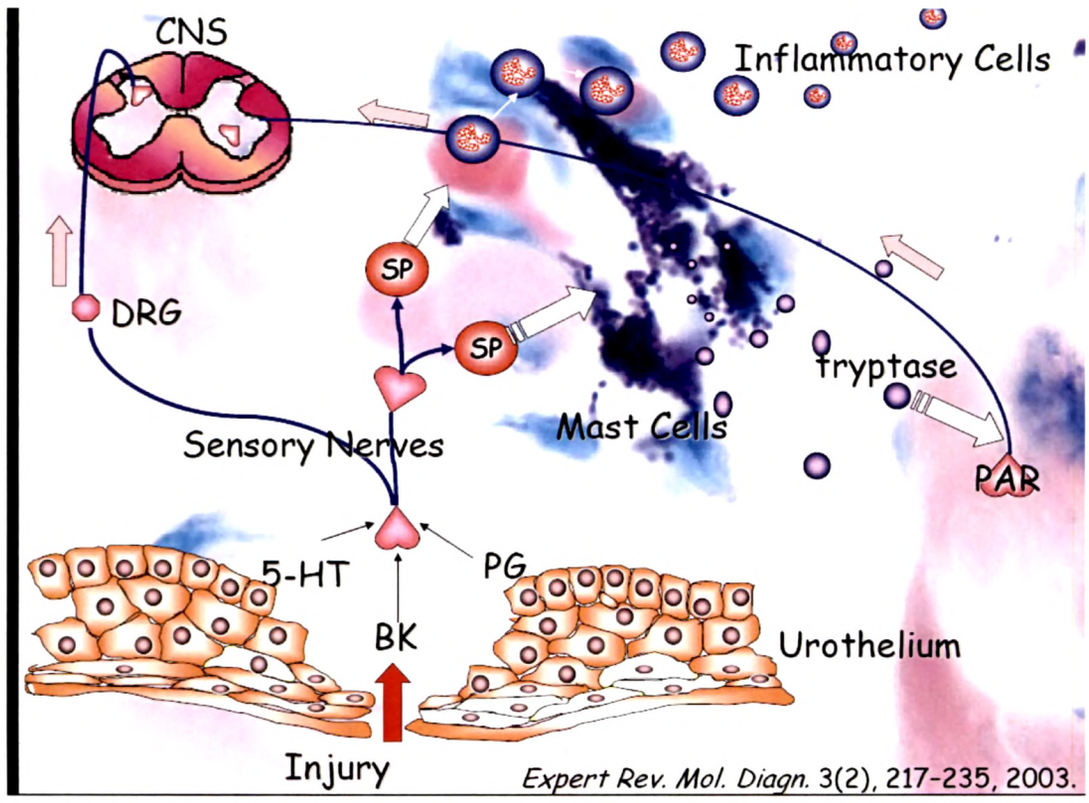


Figure 1. Inflammatory cascade

Immune responses and inflammatory responses are synonymous. When a practitioner recognizes the signs and symptoms of inflammation, he/she is witnessing the body's attempt to weaken, destroy, and isolate some injurious agent.

1.13 Types of inflammation

There are two fundamental types of inflammation: acute and chronic. Acute inflammation is characterized by a rapid onset, short duration, and profound signs and symptoms. On the other hand, chronic inflammation is characterized by a slow onset, long duration, and less obvious signs and symptoms. In addition to the two basic forms (acute and chronic), there are two others that appear less commonly: subacute and granulomatous chronic inflammation.[3] Subacute inflammation is a well defined form that has some clinical features of acute and some of chronic inflammation. Granulomatous chronic inflammation, as its name signifies, is a

special form of chronic inflammation. This type is associated with tuberculosis and some other less common diseases.

Types of Inflammation

- Acute
- Chronic
- Chronic Granulomatous
- Sub acute

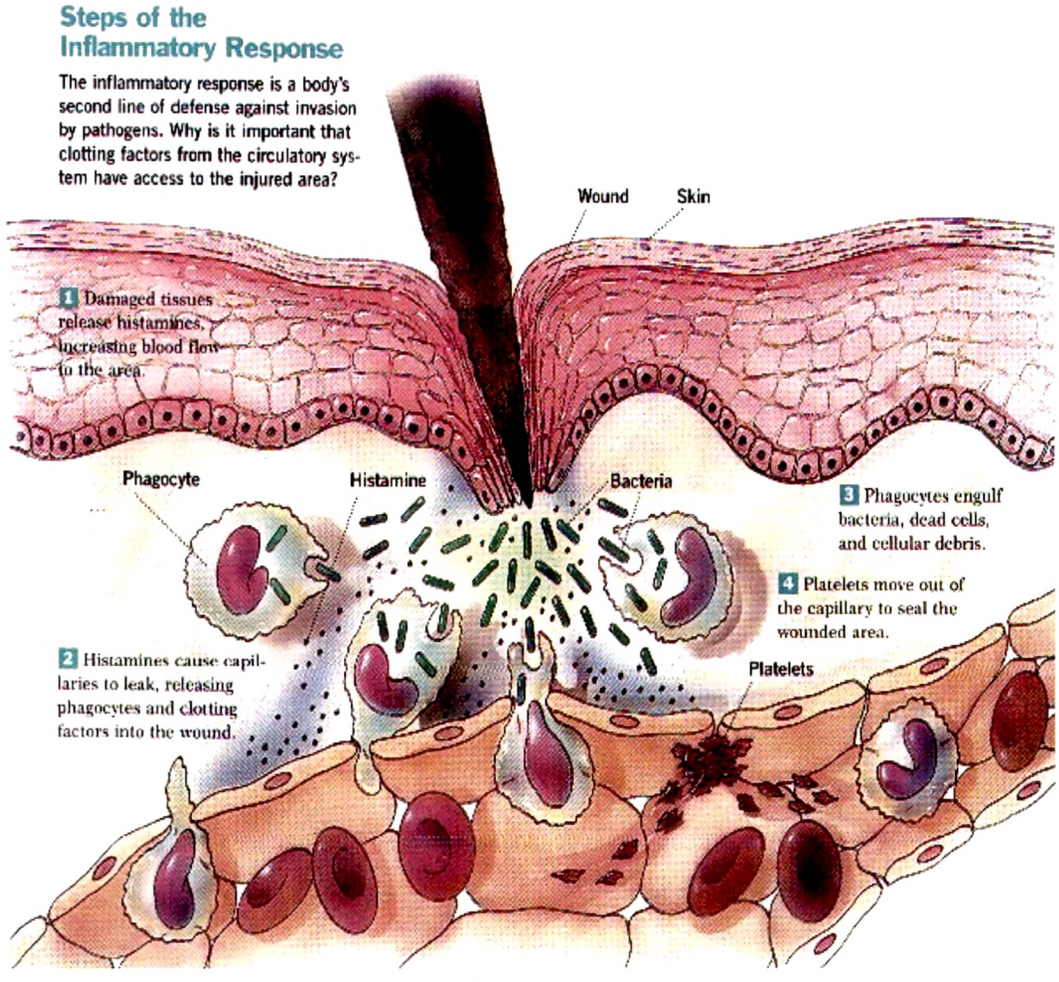


Figure 2. Inflammatory response

1.131 Acute inflammation

Acute inflammation immediately follows injury to tissues by physical, chemical, or biologic agents. The events following such injury involve blood vessel changes allowing entrance of certain blood leukocytes into the injured area. As

these cells grapple with the agent that provoked their appearance, normal surrounding tissue may be damaged or even killed. The sequences of these events have been known for decades and are not complicated to learn. More recently, however, the unfolding molecular basis for them has resulted in a maze of interacting compounds that has complicated the picture considerably. In the following discussion, microscopic and physiologic events will be emphasized more than chemical and molecular ones.

I. Sequence of events in acute inflammation

After entrance of an "injurious" (antigenic) agent into the body's connective tissue spaces, a predictable sequence of events invariably will ensue. These events occur within minutes. They explain the characteristic redness, warmth, swelling, and pain that accompany acute inflammation. In the laboratory, the first event following tissue injury is a sudden, but short-lived, contraction of small blood vessels in the immediate area. This transient vasoconstriction may be caused by stimulation of nerves in the area. Whatever its cause, it lasts for few seconds and has no apparent clinical significance.

a. Blood vessel dilation

In the first minutes, small blood vessels (capillaries and venules) increase their diameter (dilate) allowing more blood to flow into the area. This increased blood flow is fed by dilation of supplying arterioles, a process known as "active hyperemia" (hyper- = increased; -emia = blood). With increased blood flow, increased numbers of blood cells enter the area too. As more blood enters the injured area, it will be redder and warmer than surrounding unaffected areas. Celsus' terms "rubor" (red) and "calor" (heat) are often used to describe "redness" and "warmth."

b. Increased blood vessel permeability

Soon after blood vessel dilation, the blood vessels become leaky. The integrity of their lining is breached allowing the fluid portion of blood (plasma) to escape into surrounding tissues. At first, this leakage is the result of increased local blood pressure forcing a filtrate of plasma out leaving large protein molecules behind, a process known as "transudation." A short time later, changes in blood vessel lining (endothelial) cells allow even more plasma along with its important

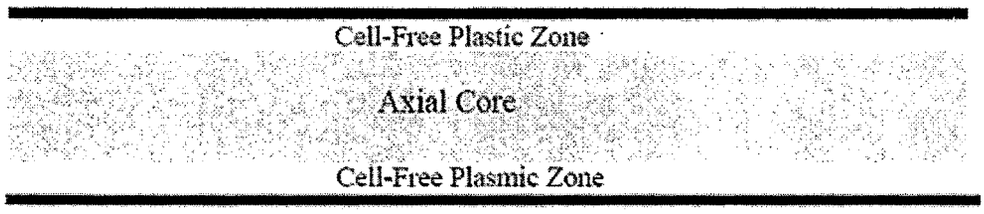
clotting and immunologic proteins to escape. The increased volume of proteins accumulating in the area of tissue injury further increases the rate of plasma escape by increasing osmotic tension. This rapid exodus of protein-rich plasma is known as "exudation."

Transudation, then, is an early short-lived event during which protein-deficient plasma exits blood vessels; in exudation, a later and longer lasting event, large amounts of protein-rich plasma leaves to accumulate in the area of tissue injury. There are two mechanisms that explain escape of plasma into the surrounding tissues in the early phase of acute inflammation: endothelial cell contraction and endothelial cell injury. The continued presence of histamine, bradykinin, and other chemical mediators causes endothelial cell contraction, an event that opens their intercellular junctions allowing early transudation of protein-deficient plasma. If the inflammatory reaction is severe and long-lasting enough, endothelial cell damage (or even death) allows rapid escape of protein-rich plasma. Such injury is caused by chemicals that accumulate in the area of tissue injury and by the activation of certain white blood cells that, in turn, secrete enzymes that in the process of destroying the inciting agent kill endothelial cells.

As might be expected, increased fluid accumulation in within the area of tissue injury produces visible swelling, or "tumor" as Celsus called it. Increased pressure within the damaged tissue and increased production of acid by-products of the inflammatory reaction causes pain ("dolor") and loss of function ("functio laesa") of the inflamed part.

c. Blood flow stagnation

Plasma leakage has an effect inside blood vessels as well. Blood cells become more closely packed (hemoconcentration) causing sluggish flow. In fact, blood flow in the affected area may even stop. When blood flow is normal, "formed elements" normally are found in a cell-rich "axial core" and are separated from the endothelial lining by a thin cell-free "plasmic zone." The maintenance of the axial core and clear plasmic zone depends on a strong rapid current of blood flow. As blood flow slows during inflammation, the axial core can no longer be maintained allowing blood leukocytes to contact the endothelial lining cells.



As blood flows rapidly through a blood vessel, the formed elements (erythrocytes, leukocytes, and platelets) are suspended in an axial core preventing their contact with endothelial lining cells. When blood flow slows and the axial core cannot not be maintained, cells may contact endothelial cells; some ricochet while others stick.

Figure 3. Diagrammatic representation of "Axial Blood Flow"

d. Margination

As blood flow slows and the axial core collapses, blood cells have the opportunity to contact the surface of endothelial cells lining the vessel wall. Some blood cells ricochet off while others stick to it. In acute inflammation, neutrophils and monocytes are sticky cells while, later, in chronic inflammation lymphocytes are the sticky ones. When blood vessels are examined with the LM at this stage of acute inflammation, neutrophils are seen lined up along the interior-lining surface, an appearance called "margination" or "pavementing." There are two explanations for adherence of leukocytes (WBC) to blood vessel walls: 1) changes in WBC and 2) changes in the endothelial lining cells. In both cases chemical mediators increase the numbers of surface receptors allowing, of course, increased adherence. C5a increases surface receptors on neutrophils. Secretions of lymphocytes and monocytes called cytokines increase the surface receptor numbers on endothelial cell surfaces.

e. Emigration

Once adherent, neutrophils and monocytes crawl along the lining surface until they find an open junction between endothelial cells. Finding a gap, they squeeze through it only to become trapped between the outer endothelial surface and the underlying basement membrane. The temporarily trapped leukocytes crawl along its basement membrane until they find a seam to squeeze through. By such considerable effort, neutrophils and monocytes leave blood vessels to enter the area of tissue injury. This active process is known as "emigration."

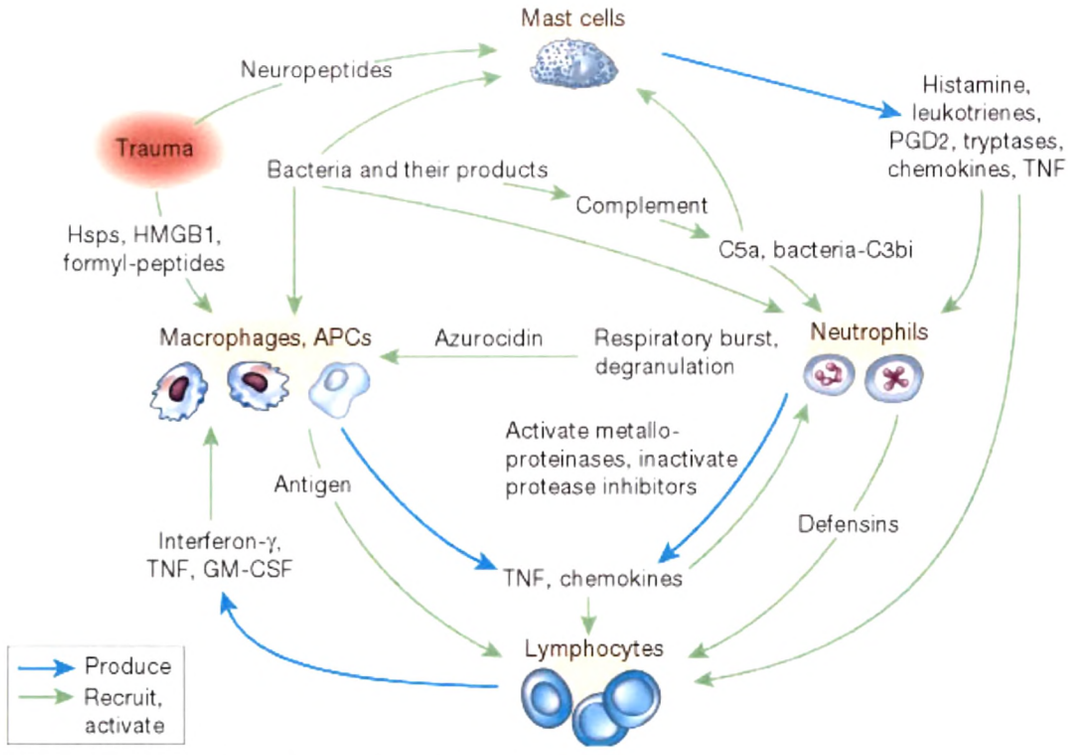


Figure 4. Cellular response in Inflammation

Neutrophils are the most common leukocyte; they compose about 65% of the circulating WBCs. These common cells soon dominate the injured area. Neutrophils die out in 48 hours or so as a consequence of self-destruction and increasing acidity of the environment. These are relatively fragile cells with a short life span. Monocytes also emigrate early in the inflammatory reaction; however, since they comprise only 5% of the circulating WBCs, the overwhelming numbers of neutrophils obscures them. Once monocytes enter the injured tissues they are given a name more indicative of their function—macrophages. Unlike neutrophils, macrophages have a long life span and have great tolerance to acidic environments. Monocytes/ macrophages outlive neutrophils and become more apparent later. As blood flows rapidly through a blood vessel, the formed elements (erythrocytes, leukocytes, and platelets) are suspended in an axial core preventing their contact with endothelial lining cells. When blood flow slows and the axial core cannot not be maintained, cells may contact endothelial cells; some ricochet while others stick.

f. Exudation

At this point in the inflammatory reaction blood plasma, neutrophils, and monocytes/macrophages have accumulated in the area of tissue injury. The term "exudate" refers to these accumulated products. The acute inflammatory exudate is composed of protein-rich plasma, of neutrophils, and monocytes/macrophages. Plasma proteins leave blood vessels early in the inflammatory response. Of these, two play a particularly important role. The first are immunoglobulins, a group of antibodies that have the ability to react with certain antigens by destroying them or by making them vulnerable to action by neutrophils and macrophages. The second are blood clotting proteins. A blood clot is composed of a meshwork of "fibrin" a protein end product of a complex interaction of plasma, tissue, and cell factors. If fibrin is produced in the area of tissue injury, it may prevent spread of the injurious agent. *Neutrophils* are capable of engulfing bacteria that have been prepared by antibodies called "opsonins." Prepared microorganisms are brought into the cell by phagocytosis, and come to lie in membrane-bound body called phagosomes. Neutrophil lysosomes fuse with phagosomes releasing powerful enzymes, hydrogen peroxide, hypochlorite, and other substances capable of killing bacteria. In the process of encountering bacteria, many neutrophils are killed. When this happens, neutrophil bactericidal agents are released into the surrounding tissue where they can kill more bacteria and some host tissue as well. *Monocytes/macrophages* appear in larger and larger numbers as neutrophils die off. These cells are a key component of a type of what immunologists call "cell based immunity." Macrophages (macro- = large; -phago = eater) are excellent phagocytes and are particularly good at engulfing and processing antigenic substances and presenting altered antigens to other cells, (lymphocytes), for ultimate destruction. Apparently neutrophils do not just appear in the injured area; rather, they are enticed to come by chemical agents. The attraction of wbcs by chemicals has been known for decades; the term "*chemotaxis*" has been used to identify it. As a chemotaxic chemical appears in the area of tissue injury, neutrophils and monocytes migrate along the path of its increasing concentration. A number of chemotaxic agents have been identified: C5a and certain leukotrienes (a product of arachidonic acid) are but two examples. These agents

bind with neutrophil and monocyte/macrophage surface receptors stimulating 1) cell movement and 2) cell activation, secretion, and degranulation.

II. Pathogenesis of acute inflammation

a. Role of the autonomic nervous system

Arterioles are "hard-wired" to the autonomic nervous system. This means that certain nerve impulses cause contraction of smooth muscle in arteriolar walls while others cause smooth muscle relaxation. Autonomic impulses play a role in relaxation of arteriole smooth muscle so that these vessels can dilate.

b. Role of chemical mediators

A host of chemicals have been identified that mediate or otherwise influence a number of responses associated with inflammation. While some of these "chemical mediators" have been known for years, others were discovered more recently. There are four classes of chemical mediators.

Sequence of Events in Acute Inflammation

1. Hyperemia
2. Increased permeability
3. Stagnation
4. Margination
5. Emigration
6. Exudation
7. Destruction of agent

Vasoactive amines (Histamine) - The first are of compounds known as "vasoactive amines." There are two important vasoactive amines—histamine and serotonin—both of which are powerful vasodilators. Histamine is found in mast cells while serotonin is found in blood platelets. Beyond its known vasodilator functions, serotonin's role in inflammation is not clear. Much more is known about histamine. It is well known, for example, that mast cell granules are histamine-filled secretory vesicles which can, when released, produce powerful dilation of blood vessels. If a lot of histamine is released all at once, a life threatening anaphylactic reaction may ensue. In run-of-the-mill inflammatory responses, however, histamine is released in

small amounts in the immediate area of tissue injury. It is in these settings that histamine acts to dilate blood vessels.

Blood Vessel Injury (Bradykinin)— These are a group of proteins constituting the "kinin system." It is the activation of this system that produces another powerful vasodilator known as "bradykinin." Initial activation results from exposure of collagen to blood plasma; such exposure is caused by injury to the
endothelial blood vessel linings allowing plasma to contact collagen in underlying basement membranes. Following collagen exposure, a series of reactions starting with activation of factor XII leads, ultimately to formation of bradykinin. It is interesting that blood vessel injury can also lead to blood clot formation by activation of a related system—the blood clotting cascade. Be that as it may, following blood vessel injury, bradykinin causes dilation of small blood vessels in the injured area.

Plasma chemicals (complement) Third, a series of plasma proteins (C1-C9) are activated by the presence of antigenic agents. These plasma proteins constitute the "complement system" or the "complement cascade." An activated form of at least one of these proteins (C5a) binds on sensitized mast cells causing them to release histamine. Because of the anaphylactic nature the release of large amounts of histamine produces, C5a and other related complement proteins are sometimes known as "anaphylatoxins."

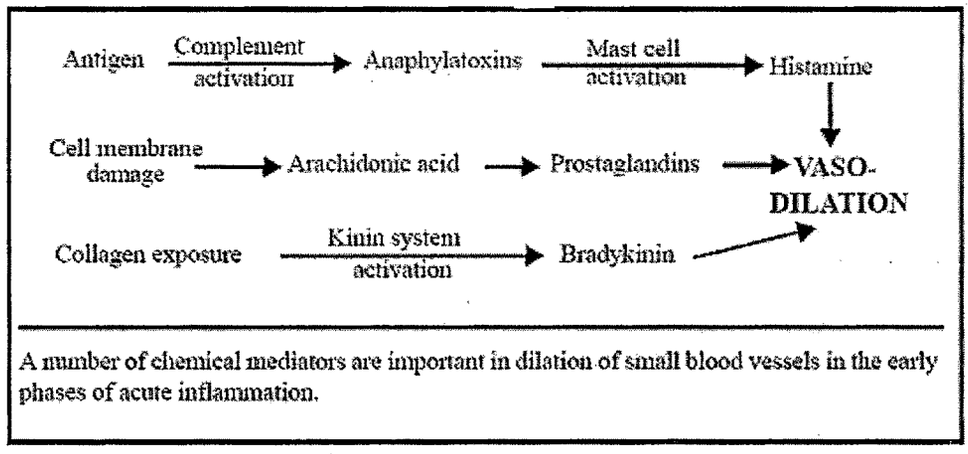
Damaged tissue cells (Prostaglandins)- Finally, the production of "prostaglandins" produces vasodilatation in the area of tissue injury. These are substances produced by a series of reactions from the damaged cell membranes and the subsequent release of "arachidonic acid." It is arachidonic acid derivatives that become the vasodilator prostaglandins.

III. Clinical features of acute inflammation

a. The "Cardinal signs" of inflammation

The presence of acute inflammation is easily recognized by its signs and symptoms. The inflamed area is red, warm, swollen, and painful. The part is so sore that the patient protects it losing its function. These features are known as the symptoms) easier by learning the terms Celsus used two millennia ago: rubor "cardinal signs" of acute inflammation. Students seem to remember these signs(and

(redness), calor (warmth), Antigen Complement Anaphylatoxins tumor (swelling), dolor (pain), and functio laesa (loss of function).[4] Any time a patient presents with a warm, red, painful swelling it is likely that their body is fighting off some bacterial infection.



A number of chemical mediators are important in dilation of small blood vessels in the early phases of acute inflammation.

Figure 5: Important chemical mediator of Inflammation

b. Systemic features of acute inflammation

Severe acute inflammatory reactions produce effects far away from the area of tissue injury. Patients with serious bacterial infections are sick. The most important of systemic changes that occur with such infections are fever and elevated white cell counts.

c. Fever

Normal body temperature (as measured orally) is 98.6° F.; in a serious infection, temperature may rise to 103–104 °C. If an infection is suspected, the patient's temperature should be measured and noted in the dental record. Fever is caused by secretion of cytokines by cells that appear in the inflammatory reaction (e.g. macrophages). Two common cytokines are interleukin-1 (Il-1) and tumor necrosis factor (TNF). Given that these factors cause fever and are produced by inflammatory cells, it follows that a large number of cells produce large amounts of cytokines resulting in higher fever. There is, then, a direct relationship between the severity of the inflammatory response and fever.

Cardinal Signs of Inflammation

English Name	Greek/Latin Name	Caused By
Redness	Rubor	Hyperemia
Warmth	Calor	Hyperemia
Swelling	Tumor	Increased Permeability
Pain	Dolor	Pressure, low pH
Loss of Function	Functio Laesa	Pain, swelling

d. Leukocytosis

In severe acute inflammatory responses, greater than normal numbers of white cells appear in circulation, a condition known as "leukocytosis" (leuko- = white; -cyt- = cell; -osis = condition of). Normally, white blood cells number about 4,000 to 10,000 in each cubic millimeter of blood in severe infections; white cell counts may reach 30,000/mm^3. The extra number of cells comes from bone marrow under the influence of the same cytokines that produce fever.

e. "Shift to the left"

Neutrophils are the most prominent cells in acute inflammation. If extraordinary numbers of these cells are needed to fight a severe infection, bone marrow is called upon to release developing neutrophils as soon as possible. As a consequence of this demand, immature neutrophils appear in the blood stream. When performing a blood count in such patients, it is possible to differentiate immature neutrophils from mature ones because the immature nuclei are not segmented, and are horse shoe-shaped. These characteristic nuclear changes have earned immature neutrophils the names "band cells" or "non-segmented neutrophils." The appearance of many immature neutrophils is sometimes designated as a "shift to the left," a reference to a form once used for reporting blood counts. By the way, neutrophils are often known as "polys" or "pmns."

f. Differential blood count

It is common practice to count various blood cell types and report the percentages of each when performing a blood count. This procedure is known as a "differential blood count." In a normal individual, neutrophils account for about 65% ,

lymphocytes for 30%, monocytes for 5%, eosinophils for 1%, and asophils for 0.5% of the WBCs. In severe acute inflammatory responses, the percentage of neutrophils (mature and immature forms) may greatly exceed the 65% occurrence rate that is normal for these cells.

g. The exudates of acute inflammation.

By common usage, the term "exudate" refers to all the materials that appear at the site of injury in inflammation. An acute inflammatory exudate is composed, then, of plasma, neutrophils, monocytes/ macrophages, fibrin, and dead surrounding tissue. However, in some circumstances one component of the exudate is common than another.

h. Purulent (suppurative) exudate

As mentioned, when an acute inflammatory reaction is severe, large numbers of neutrophils accumulate in the affected area. As the reaction proceeds, many neutrophils will die; some (or much) surrounding normal tissue will be killed too. Dead and dying neutrophils coupled with dead and dying tissue produce a foulsmelling yellow material known commonly as "pus." A more acceptable designation for this material is "purulent exudate" or "suppurative exudate." Purulent exudates are a common part of dentistry. They may exude from periodontal pockets and from deep infections that have broken through the skin or the oral mucous membrane. They may also occur at the apex of an infected tooth.

I. Serous exudate

In some exudates plasma is the predominate material that accumulates in the area of tissue injury. Clinically, plasma appears as a clear, amber-colored fluid that has no particular odor; it a "serous exudate." This exudate is found in common blisters. Burns also produce serous exudates. In fact, in large burns the exudation of plasma can be so profound that life threatening fluid loss can result.

j. Fibrinous exudate

Occasionally, fibrin is the predominate component of an exudate; if so, it is called a "fibrinous exudate." This usually occurs in the surface of some organ like the heart or lungs; it may also be seen in the pharynx or gingiva. Fibrinous exudates appear as a thick, white, shaggy covering. Sometimes the exudate is so thick that it

resembles a membrane. When this happens, the term "pseudomembrane" is used. If diphtheria, a now uncommon bacterial infection affecting the throat, should occur in a child, a pseudomembrane forming in the pharynx may block air passage causing suffocation.

IV. The lesions of acute inflammation

a. Abscesses: confined acute inflammatory lesions

An "abscess" is a localized collection of pus. Emigration of large numbers of neutrophils along with much tissue destruction is the hallmarks of abscesses. In abscesses the resulting purulent exudate is localized preventing the spread of the infectious agent elsewhere. These lesions are commonly produced by a group of microorganisms known as the pyogenic (pusproducing) bacteria. The staphylococci are a group of bacteria possessing pyogenic properties.

Treatment of an abscess involves removal of the causative agent (usually a bacterial infection). Once the microorganisms (or other causes) are dealt with it becomes necessary to remove the purulent exudate before healing can occur. If unattended, an abscess may drain spontaneously by expanding along anatomic planes until it nears a skin or mucous membrane surface. When the surface is reached, the abscess will drain, and healing will begin (if the offending microorganism is eliminated). However to prevent this, a practitioner directs drainage by cutting into the abscess and placing an artificial drain directing pus to a favorable site. This procedure is known as "incision and drainage" or an "I&D."

b. Cellulitis: the spreading acute inflammatory lesion

If pus-production if not localized but spreads into surrounding tissues instead, the resulting lesion is known as "cellulitis." The purulent exudate and the microorganism that produced it spread through anatomic planes to distant sites.

These spreading infections are serious life-threatening events. They must be treated by vigorous 1)elimination of the infectious agent (usually with antibiotics) and 2) incision and drainage of the lesion. Cellulitis is usually caused by infection with pyogenic bacteria that produce "spreading factors." The streptococci are notorious in this regard. These factors are bacterial-produced enzymes that dissolve connective

tissue ground substance (hyaluronidases) and/or inflammation-produced fibrin (fibrinolysins).

c. Ulcers: denuded acute inflammatory lesions

Inflammation occurring under an epithelial covering membrane may destroy the overlying epithelial cells. Once the covering epithelium is lost, the underlying connective tissue will be exposed. This lesion is an "ulcer." Ulcers are common lesions encountered in the oral cavity—canker sores (aphthous stomatitis) are common examples of oral ulcerations.

d. Microscopic features of acute inflammation

Acute inflammation is easily recognized by its LM appearance. As might be expected, dilated congested capillaries are prominent. Neutrophils are also an obvious feature. Therefore, these neutrophils are very specific (pathognomic) for acute inflammation. In addition to dilated/congested blood capillaries/venules and neutrophils, distended tissue spaces suggest the exudation of plasma in the area. Fibrin may be present as well. Monocytes/macrophages are present also, however the much larger numbers of neutrophils in the area obscures them.

Microscopic Features Acute Inflammation

- Dilated capillaries
- Engorged capillaries
- Neutrophils
- Distended tissue spaces
- Fibrin

III. Chronic inflammation

If inflammation has a subdued, quiet onset and lasts for days to weeks, the term "chronic inflammation" is used. This type of inflammation is, then, characterized by an insidious onset and long duration. The signs and symptoms of chronic inflammation are not as dramatic as those associated with acute inflammation.

a. Etiology and pathogenesis of chronic inflammation[5]

If an inflammatory reaction starts as acute but persists, it will enter a chronic phase. There are two general causes of such persistence: the inability to eliminate or continual reacquisition of the offending agent. These situations are common in dentistry where, for example, an open pulp chamber keeps reintroducing inflammatory/bacterial products into the tissues around the root (periapical tissues). It also may occur when there is continual exposure to some inanimate materials like pollens and dusts. More often than not, chronic inflammation arises without going through an acute phase first (de novo chronic inflammation). Two examples of this come to mind: persistent infections and autoimmune diseases. Infection with a microorganism of low virulence that cannot be eliminated easily may result in chronic rather than acute inflammation. Tuberculosis and some dental conditions (to be discussed later) are examples of such infections. Sometimes a patient may be "allergic" to her/his own cells. This condition is known as autoimmunity. In these cases, the affected patient's cells serve as a source of constant stimulation of the chronic inflammatory process. Systemic lupus erythematosus and rheumatoid arthritis are autoimmune diseases characterized by chronic inflammation.

b. The cells of chronic inflammation

The mixture of cells associated with chronic inflammation is different than the mixture associated with acute inflammation. In chronic inflammation, macrophages and lymphocytes are the predominant cells; there are few, if any, neutrophils. These, along with most other cells associated with chronic inflammation, have single nuclei. Because of this feature, they are commonly known as "mononuclear cells."

Macrophages

Macrophages are monocytes that entered an area of tissue injury. They can live for months and can thrive in acid environments. In order for macrophages to carry out their functions, they must be stimulated (activated) by chemical mediators. Among the chemical mediators are lymphokines (cytokines secreted by lymphocytes), fibronectin-coated surfaces, and mediators that initiate acute inflammation. Macrophages are excellent phagocytes. They engulf and process antigens allowing them to be neutralized by other cells (lymphocytes). Activated

macrophages can also engulf and kill certain microorganisms. Macrophages also secrete a number of substances that assist in the recruitment of other cells (monokines) and cause tissue destruction (collagenases, elastases, reactive oxygen).

T-lymphocytes

Lymphocytes emigrate from blood vessels late in an inflammatory reaction. Lymphocytes account for about one-third (33%) of the circulating leukocytes; they are the predominant cells in chronic inflammation. There are two types of lymphocytes: T and B. T lymphocytes arise from the thymus gland and are responsible for cell-based immunity. B lymphocytes, on the other hand, arise from bone marrow and are responsible for humoral immunity. T cells must be activated before they carry out their functions. Such activation is effected by monokines (secretory stimulants from monocytes [macrophages] and, in some cases, directly by antigens. Once activated, lymphocytes can react with certain antigens destroying them or rendering them harmless. They also secrete lymphokines that stimulate macrophages. Thus, macrophages and lymphocytes are interdependent—the activation of one stimulates the activation of the other.

B-lymphocytes (plasma cells)

Plasma cells are derived from activation of a class of lymphocytes known as "B cells." They do not circulate in the blood stream but are transformed in lymphoid organs or at the site of chronic inflammation. They are recognized by their off-center nuclei, abundant basophilic cytoplasms, pale spots near the nuclei (negative Golgi images), and clock-face distribution of nuclear chromatin.

Plasma cells manufacture and secrete antibodies against specific antigens. The antibodies that circulate in blood plasma are derived from plasma cells; these circulating antibodies are called "humoral antibodies." A plasma cell can only produce antibodies against a single antigen. Once a B lymphocyte is activated, it proliferates creating a clone of cells capable of producing antibodies against the antigen that stimulated it.

Eosinophils

Eosinophils are related to neutrophils; both display a segmented nucleus; both are polymorphonuclear leukocytes. Eosinophils comprise about 3% of the circulating wbcs and are recognized by the bright red granules within their cytoplasm. These granules are filled with a substance called "major basic protein" that can destroy some parasites and some cells. These cells are not seen in all chronic inflammatory reactions. Rather, they appear in parasitic infestations, hypersensitivity reactions, and some autoimmune conditions.

Multinucleated giant cells

Huge cells with many nuclei may appear in chronic inflammatory reactions. These cells are formed from the fusion of several macrophages and are called "multinucleated giant cells." They are often seen associated with foreign particulate matter (splinters, talc, debris). They may also accompany reactions to certain microorganisms of low virulence (e.g. tuberculosis).

Fibroblasts and collagen

Fibroblasts and the collagen they produce are prominent features of chronic inflammation. In fact, over exuberant collagen formation may permanently deform inflamed tissues; this circumstance in known as "fibrosis." Fibroblasts are recruited to enter an area of tissue injury by lymphokines and monokines. Once in the area, they produce collagen to replace that which has been destroyed. In the inflammatory reaction does not resolve in a reasonable time, the collagen can build up to scar tissue proportions (fibrosis).

c. The microscopic features of chronic inflammation

The presence of lymphocytes (and often, macrophages) and collagen are the two constant microscopic features of chronic inflammation. While plasma cells, eosinophils, and giant cells may appear in certain situations, lymphocytes are always present and if the reaction lasts more than a week or two collagen is always present as well. Dilated blood vessels so characteristic of acute inflammation are usually absent in chronic inflammation.

Microscopic Features of Chronic Inflammation

- Lymphocytes
- Macrophages
- Fibroblasts
- Collagen
- Plasma cells, giant cells, eosinophils

d. The clinical features of chronic inflammation

The redness, warmth, swelling, pain, loss of function, and fever associated with acute inflammation are absent or greatly suppressed in chronic inflammation.

e. Complications of chronic inflammation.

Unlike acute inflammation where the reaction itself may be life-threatening (e.g. cellulitis), the adverse effects of chronic inflammation are not so dramatic. Two complications are rather common: fibrosis and persistence.

f. Scarring in chronic inflammation

Much tissue can be destroyed during a long-standing chronic inflammatory reaction. This missing tissue is usually replaced by continual production of collagen by fibroblasts. If the inflammatory reaction persists for a long time, collagen build up can be significant. If this occurs, scars may form causing permanent distortion of the tissue and interfere with its function. Also, the presence of scar tissue may hinder regeneration of parenchymal cells.

g. Persistence of chronic inflammation

Substances with low antigenic properties may not be eliminated quickly. If these persist, the chronic inflammatory reaction may be continually stimulated for years. Similarly, reactions to one's own cells (autoimmunity) may also produce long-standing chronic inflammation due to continual cellular destruction and, therefore, the unending supply of antigen.

IV. Granulomatous chronic inflammation

Under certain circumstances a chronic inflammatory reaction will acquire features so special that they will narrow a diagnosis to a group of conditions called

"granulomatous diseases." These conditions include tuberculosis, syphilis, leprosy, and most fungal (mycotic) infections. The lesion of granulomatous chronic inflammation is the "granuloma." It is a little mass of tissue composed of chronic inflammation with a background of reparative tissue consisting of new capillaries, new fibroblasts, and new collagen. This reparative tissue is called "granulation tissue." It is the presence of granulation tissue that gives the granuloma its name.

When macrophages become activated they acquire special morphologic features. These cells acquire large, round nuclei that remind pathologists of epithelial cell nuclei. It is this feature that gives rise to their designation as "epithelioid cells." Epithelioid cells are diagnostic of granulomatous chronic inflammation.

IV Subacute inflammation

Pathologists do not speak of subacute inflammation often because it is so ill-defined that its microscopic appearance cannot be described. However, clinicians sometimes use the term to refer to a clinical situation in which the signs and symptoms displayed by the patient are neither "acute" nor "chronic"—they seem to be somewhere in between. In these cases, the reaction is neither "clinically acute" nor "clinically chronic."

1.2 Non-steroidal anti-inflammatory drugs(NSAIDs)

Non-steroidal anti-inflammatory drugs, usually abbreviated to NSAIDs, are drugs with analgesic, antipyretic and anti-inflammatory effects - they reduce pain, fever and inflammation. The term "non-steroidal" is used to distinguish these drugs from steroids, which (among a broad range of other effects) have a similar eicosanoid-depressing, anti-inflammatory action. As analgesics, NSAIDs are unusual in that they are non-narcotic. NSAIDs are sometimes also referred to as non-steroidal anti-inflammatory agents/analgesics (NSAIAs) or non-steroidal anti-inflammatory medicines (NSAIMs). The most prominent members of this group of drugs are aspirin, ibuprofen, and naproxen partly because they are available over-the-counter in many areas. Paracetamol (acetaminophen) has negligible anti-inflammatory activity, and is strictly speaking not an NSAID. NSAIDs are commonly prescribed for long-term management of rheumatoid arthritis, osteoarthritis and other rheumatic diseases. NSAIDs do not alter the progression of the disease, but provide considerable relief from the symptoms of arthritis. Unfortunately the risks of NSAIDs are higher than previously thought. Many studies on long-term NSAID use have indicated a 2 to 4% annual incidence of serious gastrointestinal complication requiring hospitalization.[7,8] It has also been established that 30% of long-term NSAID users will develop an ulcer at least once.[7] This report explains how NSAIDs work, why they cause gastrointestinal side effects, and describes new ways of preventing the problems they cause.

Beginning in 1829, with the isolation of salicin from the folk remedy willow bark, NSAIDs have become an important part of the pharmaceutical treatment of pain (at low doses) and inflammation (at higher doses). Part of the popularity of NSAIDs is that, unlike opioids, they do not produce sedation or respiratory depression and have a very low addiction rate. NSAIDs, however, are not without their own problems (see below). Certain NSAIDs including ibuprofen and aspirin, have become accepted as relatively safe and are available over-the-counter without prescription and are available over-the-counter without prescription.

The word arthritis means entirely different things to different people. To many it is the wearing of joints in the elderly with osteoarthritis. Others think of the swollen and painful joints of younger people with rheumatoid arthritis. In actuality, arthritis is a general term that means "inflammation of the joint", a characteristic of over 100 distinct rheumatic diseases. Canada, a country with a population of just under 30 million people, has more than 3.7 million people diagnosed with some form of arthritis. Arthritis ranks in the country's top three chronic disorders along with allergies and heart disease. It results in 22 million working days lost annually at a cost of $1.1 billion, and is the primary cause of disability for over 600 000 Canadians.[9]

Despite the prevalence of arthritis, the full mechanism of the disease process remains unknown. Many rheumatic diseases are thought to be a type of autoimmune disease, a disorder where the immune system loses the ability to distinguish between normal healthy tissue and foreign invaders. For example, in rheumatoid arthritis, immune system cells attack the body's joint linings. Treatment of arthritis is limited to preventing disease progression and managing the symptoms.

1.21 NSAIDs in the treatment of arthritis

The traditional treatment approach for rheumatoid arthritis (Figure 6) starts with nonsteroidal anti-inflammatory drugs to control symptoms. A balance of exercise and rest maintains flexibility and range of motion. NSAIDs can also help by removing the pain barrier to exercise. Slow-acting anti-rheumatic drugs (SAARDs) like methotrexate are introduced later in the disease process. This approach was used because rheumatoid arthritis was thought to be a benign disease controllable by NSAIDs, with SAARDs being too toxic for regular use.[10]

It is now recognized that rheumatoid arthritis causes significant morbidity and mortality, and NSAIDs do not prevent the progression of the disease. In fact, slow-acting anti-rheumatic drugs like sulfasalazine and the anti-malarial drugs are considered to be no more toxic than many NSAIDs. This knowledge has led many physicians to start using SAARDs earlier in an arthritis treatment program.

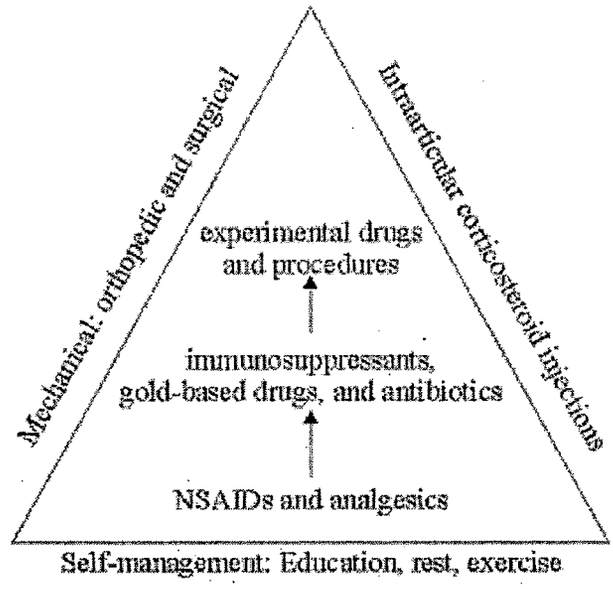


Figure 6: Traditional treatment pyramid for Rheumatoid Arthritis

Although they do not change the underlying disease process, nonsteroidal anti-inflammatory drugs provide considerable improvement in daily life for people suffering from arthritis and other rheumatic diseases. They are also used for sports injuries, chronic low back pain, menstrual pain and headaches. Probably the most important benefit to using an NSAID for a rheumatic disease is that it reduces symptoms of pain and inflammation, allowing beneficial exercise and physiotherapy. All together, NSAIDs are used daily by approximately 30 million people worldwide, constituting a world market in excess of $2 billion.[11] Nearly every person with arthritis takes an NSAID regularly for many years, so it comes as no surprise that NSAIDs are often marketed as 'arthritis medication'.

Nonsteroidal anti-inflammatory drugs can be broken down into five different classes: acetylsalicylic acid, acetic acids, fenamates, oxicams and propionic acids. These classes and some examples of individual drugs are summarized in Table 1.

Table 1: NSAID Classes Below is a comprehensive list of different types of NSAIDs.

Types of NSAIDs

Generic name	**Brand name(s)**
Salycylic acids	
Aspirin (acetylsalicylic acid)	Ascriptin, Bayer, Ecotrin
Choline magnesium trisalicylate	Trilisate
Diflunisal	Dolobid
Salsalate	Disalcid, Salflex
Propionic acids	
Fenoprofen	Nalfon
Flurbiprofen	Ansaid
Ibuprofen	Advil, Motrin, Nuprin
Ketoprofen	Actron, Orudis, Oruvail
Naproxen	Aleve, Anaprox, Naprelan, Naprosyn
Oxaprozin	Daypro
Acetic acids	
Diclofenac	Cataflam, Voltaren
Indomethacin	Indocin
Sulindac	Clinoril
Tolmetin	Tolectin
Enolic acids	
Meloxicam	Mobic
Piroxicam	Feldene, Fexicam
Fenamic acids	
Meclofenamate	Meclomen

Mefenamic acid	Ponstel
Napthylalkanones	
Nabumetone	Relafen
Pyranocarboxylic acids	
Etodalac	Lodine
Pyrroles	
Ketorolac	Toradol
COX-2 inhibitors	
Celecoxib	Celebrex
Valdecoxib	Bextra *(withdrawn from market in 2005)*
Rofecoxib	Vioxx *(withdrawn from market in 2004)*

1.22 Mechanism of action of NSAIDs

1.22.1 The Cyclooxygenase pathway

Nonsteroidal anti-inflammatory drugs work by interfering with the cyclooxygenase pathway (Figure 7). The normal process begins with arachidonic acid, a dietary unsaturated fatty acid obtained from animal fats. This acid is converted by the enzyme cyclooxygenase to synthesize different prostaglandins. The prostaglandins go on to stimulate many other regulatory functions and reactionary responses in the body. Recent research[12,13,14] has shown that there are two types of cyclooxygenase, denoted COX-1 and COX-2. Each type of cyclooxygenase lends itself to producing different types of prostaglandins.

Different mechanisms stimulate the two types of cyclooxygenase. COX-1 is stimulated continuously by normal body physiology. The COX-1 enzyme is constitutive, meaning that its concentration in the body remains stable. It is present

in most tissues and converts arachidonic acid into prostaglandins. These prostaglandins in turn stimulate normal body functions, such as stomach mucus

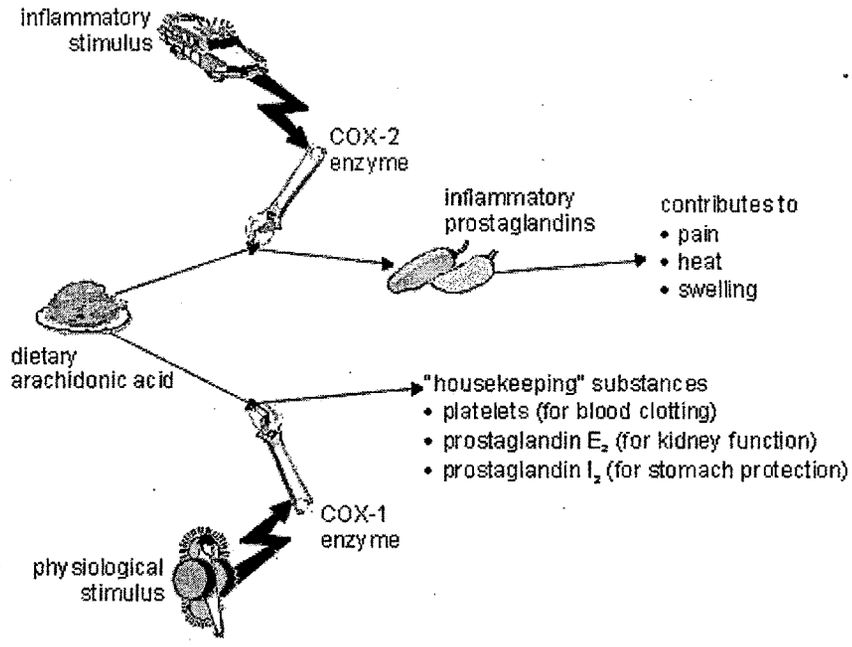


Figure 7: The cyclooxygenase pathway

production and kidney water excretion, as well as platelet formation. The location of the COX-1 enzyme dictates the function of the prostaglandins it releases.[13] For example, COX-1 in the stomach wall produces prostaglandins that stimulate mucous production. In contrast, the COX-2 enzyme is induced. It is not normally present in cells but its expression can be increased dramatically by the action of macrophages, the scavenger cells of the immune system.[12] COX-2's most important role is in inflammation. COX-2 is involved in producing prostaglandins for an inflammatory response. COX-1 is stimulated continually, and COX-2 is stimulated only as a part of an immune response.

1.22.2 Inflammatory role of prostaglandin

Inflammation is a nonspecific response of the immune system to damaged cells. It is characterized by redness, pain, heat and swelling of tissue. Although there are many components to inflammation, only the prostaglandin component is

substantially reduced by the action of an NSAID. Prostaglandin E2 (PGE2) is the form of prostaglandin most associated with inflammation.[12] It dilates blood vessels, allowing more blood to flow through the affected tissue. The increased blood flow generates the heat and redness of inflammation. PGE_2 prolongs pain, and may also stimulate the emigration of phagocytes through capillary walls. In an autoimmune disease, the body is inadvertently attacking its own cells through the inflammatory process as though they were foreign particles. So, by reducing prostaglandin, the swelling, heat and pain of inflammation is reduced. However, other parts of the inflammatory response, such as the destruction of healthy tissue by phagocytes, continue unabated in an autoimmune disease.

1.22.3 Action of NSAIDs on cyclooxygenase

The two forms of cyclooxygenase have equal molecular weights and are very similar in structure (Table 2). However, the attachment site of COX-1 is smaller than the attachment site of COX-2, so it accepts a narrower range of structures as substrates.[12]. NSAIDs work by temporarily blocking the attachment site for arachidonic acid on the cyclooxygenase enzyme, preventing the enzyme from converting arachidonic acid to prostaglandin. The exception is aspirin, which irreversibly acetylates cyclooxygenase.[13] It takes longer for the effects of aspirin to wear off because new enzymes must be formed to replace the altered enzymes. When COX-1 is acetylated by aspirin, the site for arachidonic acid is blocked. However, when aspirin acetylates COX-2 the active site is still large enough to accept arachidonic acid.[12] Acetylation also accounts for aspirin's anti-platelet effect, which helps to prevent blood clots and the chance of a heart attack, although use of aspirin for this purpose has been recently questioned.[15] A significant anti-platelet effect requires just 75 mg of aspirin a day, in comparison with 4000 mg of aspirin a day to obtain an anti-inflammatory effect. Other NSAIDs also affect platelet production, but the effect is much milder. Interestingly, acetaminophen has only mild activity on COX-1 and COX-2, yet it manages to reduce prostaglandin synthesis in the brain, relieving pain and fever, as does aspirin and the NSAIDs. Perhaps

acetaminophen is more specific to a third form of cyclooxygenase, a 'COX-3', which exists in the brain[12], accounting for the drug's analgesic and antipyretic abilities.

Table 2. Comparison of COX-1 and COX-2

Parameter	COX-1	COX-2
Regulation	Usually Constitutive	Inducible
Range of Induced Gene Expression	2 to 4-fold	10 to 80-fold
Rate of Gene Activation	24 hours	0.5 to 4 hours
Effect of Glucocorticosteroids	Little or None	Inhibits Expression
Relative Size of Active Site	Smaller	Larger
Rate of Arachidonic Acid Consumption	34 nmol/min/mg	39 nmol/min/mg
Effect of aspirin on COX activity	Inhibited	Not Affected

Some NSAIDs have worse side effects than others, although they have the same amount of anti-inflammatory action. This is due to the specificity each drug has towards each form of COX. Most NSAIDs inhibit COX-1 more than COX-2. When NSAIDs are ordered by their COX-1 to COX-2 specificity ratio (Figure 8), the drugs with the greatest specificity to COX-1 also happen to be the drugs with the greatest side effects. For example, aspirin is about 160 times more specific to COX-1 than COX-2, and is also well known for its ulcerative potential. Other drugs with high gastrointestinal side effects are sulindac, tolmetin and piroxicam. Piroxicam is the most dangerous drug of its class, with a COX-1 to COX-2 specificity ratio of 250:1. In fact, piroxicam carries a substantially greater risk than any other NSAID. One study reported almost twice as many serious gastrointestinal reactions for piroxicam than for any other NSAID. Piroxicam carries an unacceptably greater risk without additional benefit, and should not be used when less toxic NSAIDs are available.

Current data on specificity ratios is imprecise and highly dependent on the measurement method used. However, this chart still illustrates the general trend: piroxicam, tolmetin and aspirin are more COX-1 specific and more likely to cause

gastrointestinal side effects than meloxicam, naproxen and diclofenac. In summary, NSAIDs block both forms of cyclooxygenase from converting arachidonic acid to prostaglandins. The benefit of an NSAID comes from its COX-2 blocking action, keeping COX-2 from forming prostaglandins involved in inflammation. The undesirable side effects of NSAIDs result from COX-1 blocking. Each NSAID affects COX-1 and COX-2 differently, and those that have the highest ratio of COX-1 to COX-2 specificity are also the drugs with the greatest number of side effects. The recent knowledge on cyclooxygenase specificity will lead to the development of new COX-2-specific NSAIDs.

1.22.4 COX-2 inhibitors

COX-2 selective inhibitor is a form of Non-steroidal anti-inflammatory drug (NSAID) that directly targets COX-2, an enzyme responsible for inflammation and pain. Selectivity for *COX-2* reduces the risk of peptic ulceration, and is the main feature of celecoxib, rofecoxib and other members of this drug class. Cox-2-selectivity does not seem to affect other adverse-effects of NSAIDs (most notably an increased risk of renal failure), and some results have aroused the suspicion that there might be an increase in the risk for heart attack, thrombosis and stroke by a relative increase in thromboxane. Rofecoxib was taken off the market in 2004 because of these concerns.

a. Discovery of COX-2

The COX-2 enzyme was discovered in 1988 by Daniel Simmons, a Brigham Young University researcher formerly of Harvard University. Dr. Simmons immediately understood the importance of his discovery. The same day the enzyme was sequenced, he had his notebook notarized as proof of his discovery. Subsequently, Monsanto, the research firm with whom Dr. Simmons had contracted, fraudulently broke contract and refused to give Dr. Simmons any royalties and profits from his discovery. A lawsuit is currently in progress by Dr. Simmons against the drug developers.

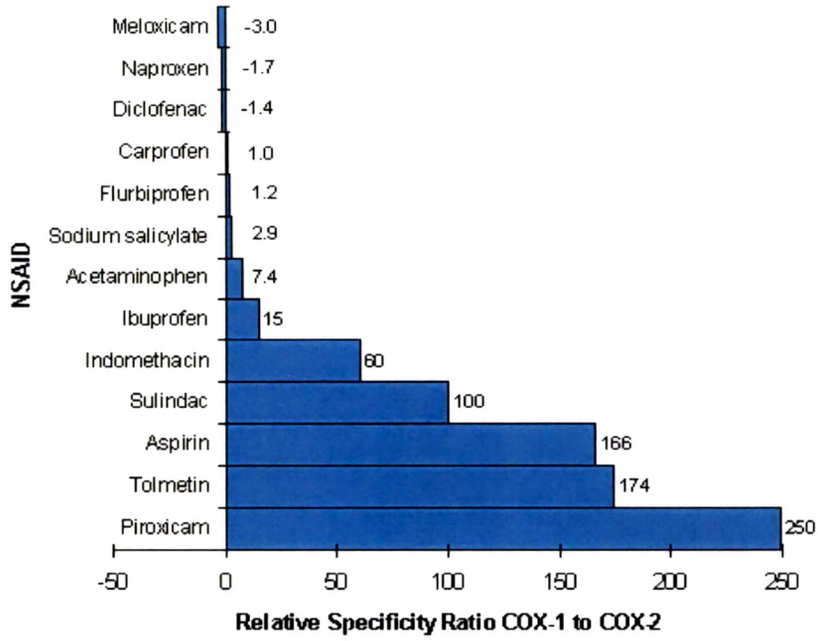


Figure 8. Cyclooxygenase specificity of common NSAIDs.

In the course of the search for a specific inhibitor of the negative effects of prostaglandins which spared the positive effects, it was discovered that prostaglandins could indeed be separated into two general classes which could loosely be regarded as "good prostaglandins" and "bad prostaglandins", according to the structure of a particular enzyme involved in their synthesis, cyclooxygenase Prostaglandins whose synthesis involves the cyclooxygenase-I enzyme, or COX-1, are responsible for maintenance and protection of the gastrointestinal tract, while prostaglandins whose synthesis involves the cyclooxygenase-II enzyme, or COX-2, are responsible for inflammation and pain.[16]

The existing nonsteroidal anti-inflammatory drugs (NSAIDs) differ in their relative specificities for COX-2 and COX-1; while aspirin is equipotent at inhibiting COX-2 and COX-1 enzymes *in vitro* and ibuprofen demonstrates a sevenfold greater inhibition of COX-1, other NSAIDs appear to have partial COX-2 specificity, particularly meloxicam (Mobic). Studies of meloxicam 7.5 mg per day for 23 days find a level of gastric injury similar to that of a placebo, and for meloxicam 15 mg per

day a level of injury lower than that of other NSAIDs; however, in clinical practice meloxicam can still cause some ulcer complications.

A search for COX-2-specific inhibitors resulted in promising candidates such as valdecoxib, celecoxib, and rofecoxib (marketed under the brand names Bextra, Celebrex, and Vioxx respectively). Valdecoxib and rofecoxib are about 300 times more potent at inhibiting COX-2, than COX-1, suggesting the possibility of relief from pain and inflammation, without gastrointestinal irritation, and promising to be a boon for those who had experienced such adverse effects previously or had comorbidities that could lead to such complications.

Celecoxib is approximately 30 times more potent at inhibiting COX-2 than COX-1. Although individual reactions to particular NSAIDs vary, in general the efficacy of COX-2 inhibitors has proved similar to that of other NSAIDs, as expected since both classes of drug inhibit the desired target, the action of COX-2 prostaglandins. The drug's effectiveness is similar to that of traditional NSAIDs such as ibuprofen, diclofenac, or naproxen.

b. Adverse-effects and withdrawal of Vioxx

On September 27, 2004 Vioxx (Rofecoxib) was withdrawn voluntarily from the market, due to an increased risk of myocardial infarction and stroke. At present it is unclear whether this adverse effect pertains also to other drugs of this group or is specific for Vioxx. Beasley Allen Law Firm is spearheading the review of over 31,000 claims against the manufacturers of Bextra, Celebrex and Vioxx.

c. Early COX-2 inhibiting drugs

Celebrex and Vioxx were introduced in 1999 and rapidly became the most frequently prescribed new drugs in the United States. By October 2000, their US sales exceeded 100 million prescriptions per year for $3 billion, and were still rising, sales of Celebrex alone reaching $3.1 billion in 2001. A Spanish study found that between January 2000 and June 2001, 7% of NSAID prescriptions and 29% of

NSAID expenditures were for COX-2 inhibitors. Over the period of the study, COX-2 inhibitors rose from 10.03% of total NSAIDs prescribed by specialty physicians to 29.79%, and from 1.52% to 10.78% of NSAIDs prescribed by primary care physicians (98.23% of NSAIDs and 94.61% of COX-2 inhibitors were prescribed by primary care physicians). For specialty physicians, rofecoxib and celecoxib were third and fifth most frequently prescribed NSAIDs but first and second in cost, respectively; for primary care physicians they were ninth and twelfth most frequently prescribed NSAIDs and first and fourth in cost.

The cause of the rapid widespread acceptance of Celebrex and Vioxx by physicians was the publication of two large trials in JAMA, the Celecoxib Long-term Arthritis Safety Study (CLASS) study, and the Vioxx Gastrointestinal Outcomes Research (VIGOR) study. Both publications concluded that COX-2 specific NSAIDs were associated with significantly fewer adverse gastrointestinal effects. In the CLASS trial comparing Celebrex 800 mg/day to ibuprofen 2400 mg/day and diclofenac 150 mg/day for osteoarthritis or rheumatoid arthritis for six months, Celebrex was significantly associated with fewer upper gastrointestinal complications (0.44% vs. 1.27%, P=0.04), with no significant difference in incidence of cardiovascular events in patients not taking aspirin for cardiovascular prophylaxis. In the VIGOR trial testing Vioxx 50 mg/day versus naproxen for rheumatoid arthritis, Vioxx reduced the risk of symptomatic ulcers and clinical upper gastrointestinal events (perforations, obstructions and bleeding) by 54%, to 1.4% from 3%, the risk of complicated upper gastrointestinal events (complicated perforations, obstructions and bleeding in the upper gastrointestinal tract) by 57%, and the risk of bleeding from anywhere in the gastrointestinal tract by 62%. An enormous marketing effort capitalized on these publications; Vioxx was the most heavily advertised prescription drug in 2000, and Celebrex the seventh, according to IMS Health.

d. Comparative studies

In a metaanalysis of eight osteoarthritis studies, the incidence of withdrawal because of adverse gastrointestinal events was 3.5% for Vioxx, compared to 4.8% for ibuprofen, diclofenac, or nabumetone (Relafen). Endoscopic studies of patients receiving Celebrex 50-400 mg twice daily for 12-24 weeks found rates of upper gastrointestinal complications similar to placebo and significantly lower than naproxen 500 mg twice daily and ibuprofen 800 mg three times daily, but not statistically significantly different from patients receiving diclofenac 75 mg twice daily.11 The analysis found that Vioxx provided significant gastrointestinal benefits in patients both at high risk and at low risk of developing gastrointestinal problems; patients at low risk still had 88% fewer gastrointestinal problems with Vioxx.[17]

The results of the CLASS study were confirmed by the Successive Celecoxib Efficacy and Safety Studies (SUCCESS) study, which examined the effectiveness and safety of celecoxib 200 mg and 400 mg daily and how well it was tolerated by patients in terms of adverse effects, compared with the most common NSAID regimens in the countries studied (diclofenac 100 mg daily and naproxen 1000 mg daily). SUCCESS showed that celecoxib was as effective as the conventional NSAIDs in controlling the pain of arthritis, and caused fewer gastrointestinal ulcers or ulcer complications (such as perforations or bleeding) and fewer upper gastrointestinal adverse effects, e.g. 29% less chance of having nausea and 22% less chance of abdominal pain. In addition, hospitalization rates for upper gastrointestinal problems were 2 to 4 times lower with celecoxib, and because there were fewer adverse effects, there was 23% less chance of a celecoxib patient stopping treatment. The study also found that there was no real advantage to taking a bigger dose of celecoxib: the 200 mg dose was found to be just as effective as the 400 mg dose.

The VIGOR study was followed by the Assessment of Difference between Vioxx and Naproxen to Ascertain Gastrointestinal Tolerability and Effectiveness (ADVANTAGE) study, which showed that 9.1% of people taking Vioxx received a

gastro-protective medicine compared with 11.2% of people taking naproxen, a reduction of 19%. In addition, after 3 months, 5.9% of people stopped taking Vioxx compared with 8.1% who stopped taking naproxen, a reduction of 27%. ADVANTAGE was the first study comparing the gastrointestinal tolerability of Vioxx and naproxen in a group that included patients taking low-dose aspirin for cardiovascular reasons. This was followed by the Experience with Vioxx in Arthritis (EVA) survey of 5,986 Belgian physicians and 74,192 people with osteoarthritis, which found that, after 12.5 or 25 mg of Vioxx once daily for 30 days, 80% of the patients wished to continue treatment with Vioxx and more than 80% of doctors said they would continue prescribing Vioxx. The preference to continue taking Vioxx was especially strong in people who previously treated with older NSAIDs.

In a six week long study comparing Vioxx 25 mg once daily, Vioxx 12.5 mg once daily, Celebrex 200 mg once daily, and paracetamol 1,000 mg four times daily for osteoarthritis of the knee, higher dose Vioxx was found to be superior to the other three treatments for reduction of nocturnal pain, and superior to Celebrex and acetaminophen for reduction of resting pain. At six weeks, 60% of high dose Vioxx patients reported a good or excellent response, compared to 46% of Celebrex patients and 39% of paracetamol patients. Low dose Vioxx was not found to be statistically significant from Celebrex at this dose. Similar results were found for early response to therapy.

However, when the Food and Drug Administration (FDA) later presented more complete data from the CLASS and VIGOR trials on its web site, the results were less certain. The CLASS trial was revealed to also have twelve and fifteen month time points which had not been discussed in the JAMA publication; in this segment of the trial, the number of ulcer-related complications for Celebrex caught up to the control NSAID group. Similarly, the complete VIGOR study data revealed that in fact, when all adverse events, not just gastrointestinal, were tabulated, the patients receiving VIOXX had suffered (barely) significantly higher incidence of adverse events overall than the control NSAID group. In particular, the risk of serious cardiovascular thrombotic events, e.g. myocardial infarction, was 1.7% in the

VIOXX patients versus 0.7% in the control group, and there were significantly more withdrawals in the Vioxx group for causes including hypertension, edema, hepatotoxicity, heart failure, or pathological laboratory findings. The mean increases in systolic and diastolic blood pressure in the Vioxx group were 4.6 mmHg and 1.7 mmHg respectively, compared to 1.0 and 0.1 mmHg in the control NSAID group. An estimated 43,000,000 Americans, nearly one out of six, suffers from arthritis. However, 42% (18 million) of these also suffer from hypertension. Therefore, the promise of better patient outcomes and lowered medical costs from use of COX-2 inhibitors may not be as great as previously hoped. Questions remain regarding the relative safety and cost effectiveness of this new class. While endoscopic evidence of gastrointestinal damage is frequently seen in studies of nonspecific NSAIDs, the actual incidence of clinically evident symptoms and patient discomfort is much lower; furthermore, in cases of short-term therapy, any such damage generally reverses itself quickly after termination of the drug.

e. Risks and adverse effects

This cardiovascular risk of COX-2 specific inhibitors is not surprising since prostaglandins are involved in regulation of blood pressure by the kidneys. Therefore, cardiovascular effects of NSAIDs prescribed for arthritis pain and inflammation need to be considered when choosing the appropriate medication for each patient[18]. A French study of osteoarthritis patients over 65 years of age determined that, compared to Celebrex (200 mg once daily), patients taking Vioxx (25 mg once daily) suffered a two-fold increase in clinically significant edema and 60% more frequent increases in systolic blood pressure greater than 20 mmHg, as early as the second week of treatment. This has significant implications, since it has been estimated that every 2 mmHg increase in blood pressure raises the risk of stroke by two thirds and the risk of myocardial infarction by one third, suggesting that Celebrex may be a better choice for hypertensive patients or those at risk for edema. In addition, COX-2 inhibitors lack some of the platelet inhibiting properties of aspirin and other nonspecific NSAIDs and may, directly or indirectly, lead to increased risk of thrombosis, particularly in high risk patients where low dose aspirin therapy is

warranted. On the other hand, this property makes them a better choice for perisurgical pain management, where inhibition of blood clotting would be problematic.

There are other differences between Celebrex and Vioxx that influence prescribing practices. Patients with known sensitivity to sulfa drugs are likely to be sensitive to Celebrex as well, due to similarity in structure. Vioxx has a more rapid onset and is approved for acute pain as well as osteoarthritis, while Celebrex is approved for rheumatoid arthritis as well as osteoarthritis.Future of Cox 2 Inhibitors a treatment for neuroblastomas.

Recent studies have shown that small tumors of the sympathetic nervous system (neuroblastoma) have abnormal levels of COX-2 expressed. These studies report that and overexpression of the COX-2 enzyme has an adverse effect on the tumor suppressor, p53. p53 is an apoptosis transcription factor normally found in the cytosol, when cellular DNA is damaged beyond repair, p53 is transported to the nucleus where is promotes p53 mediated cell suicide (apoptosis). Two of the metabolites of COX-2, prostaglandin A2 (PGA2) and A1 (PGA1), when present in high quantities binds to p53 in the cytosol and inhibits its ability to cross into the nucleus. This essentially sequesters p53 in the cytosol and prevents apoptosis. coxibs such as CELEBREX® (celecoxib), by selectively inhibiting the overexpressed COX-2, allow p53 to work properly. Functional p53 allows DNA damaged neuroblastoma cells to commit suicide through apoptosis, halting tumor growth. COX-2 up-regulation has also been linked to the phosphorylation and activation of the E3 ubiquitin ligase HDM2, a protein that mediates p53 ligation and tagged destruction, through ubiquitination. The mechanism for this neuroblastoma HDM2 hyperactivity is unknown. Studies have shown that COX-2 inhibitors block the phosphorilation of HDM2 preventing its activation. *In vitro*, the use of COX-2 inhibitors such as CELEBREX® (celecoxib) lowers the level of active HDM2 found in neuroblastoma cells. The exact process of how COX-2 inhibitors block HDM2 phosphorilation is unknown, but this mediated reduction in active HDM2 concentration level restores the cellular p53 levels. After treatment with

CELEBREX® (celecoxib), the restored p53 function allows DNA damaged neuroblastoma cells to commit suicide through apoptosis reducing the size of growth of the tumor.

1.22.5 Combinations of drugs

A model comparing the theoretical relative frequency of gastrointestinal adverse effects and cost effectiveness of celecoxib, nonspecific NSAIDs alone, NSAIDs plus a proton pump inhibitor, NSAIDs plus an H_2 receptor antagonist, NSAIDs plus misoprostol, and diclofenac/misoprostol, found the lowest probability of adverse gastrointestinal events for celecoxib, followed by NSAIDs plus a proton pump inhibitor, NSAIDs plus an H_2 receptor antagonist, NSAID plus misoprostol, diclofenac/misoprostol, and NSAID alone. In total cost, including drug plus treatment of any gastrointestinal effects, the lowest cost treatment was celecoxib, followed by NSAIDs alone and diclofenac/misoprostol, with the other NSAID plus gastrointestinal protection regimens being much more costly. Similarly, a model of cost effectiveness of rofecoxib and celecoxib compared to high-dose acetaminophen or ibuprofen, with and without misoprostol, in patients with osteoarthritis of the knee found that acetaminophen had the lowest cost for average patients. For those not responding to paracetamol, ibuprofen was the most cost effective treatment by a large margin, but for those who did not respond to acetaminophen and had a high risk of gastrointestinal damage, rofecoxib was the most cost effective treatment.

1.23 NSAID side effects

Side effects to NSAIDs vary from person to person. Common side effects to all NSAIDs are abdominal pain, diarrhea, nausea, and fluid retention. These side effects are natural, expected and unavoidable, and occur in about 30% of people taking NSAIDs.[19] Some people may find that they are allergic to a particular class of NSAIDs, or that a certain NSAID is not effective in relieving pain and inflammation. It is not uncommon for a person with arthritis to try several different NSAIDs before finding one that is effective and without annoying side effects.

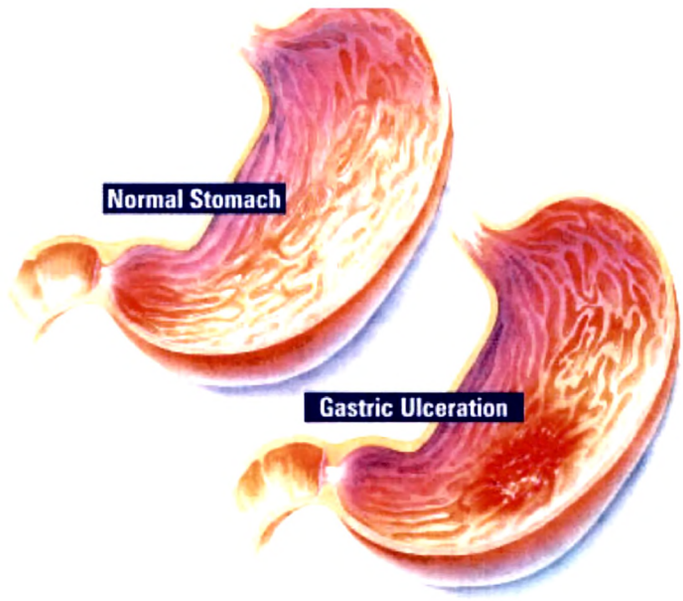


Figure 9: Stomach Ulceration

Other more serious side effects are gastrointestinal ulcers and bleeding (Figure 9), and less frequently, kidney and liver damage. An ulcer is an erosion of tissue, somewhat circular in shape. We are familiar with small, stinging mouth ulcers caused by viruses. Unfortunately, we may not feel stomach ulcers when taking NSAIDs because of the analgesic effect of these drugs. The occurrence of adverse gastric side effects is more common in aspirin and piroxicam. Other NSAIDs such as diclofenac and ibuprofen are less likely to cause ulcers or bleeding. New understanding of how NSAIDs work has finally shown why certain nonsteroidal anti-inflammatory drugs have more serious side effects than others, and has stimulated development of new NSAIDs that will greatly reduce the potential of developing dangerous side effects.

1.23.1 Stomach Self-Protection: During digestion, food moves from the mouth down the esophagus into the stomach. The stomach adds hydrochloric acid and pepsin enzyme. From the stomach, food passes into the duodenum, where digestion and nutrient absorption continue (Figure 10).

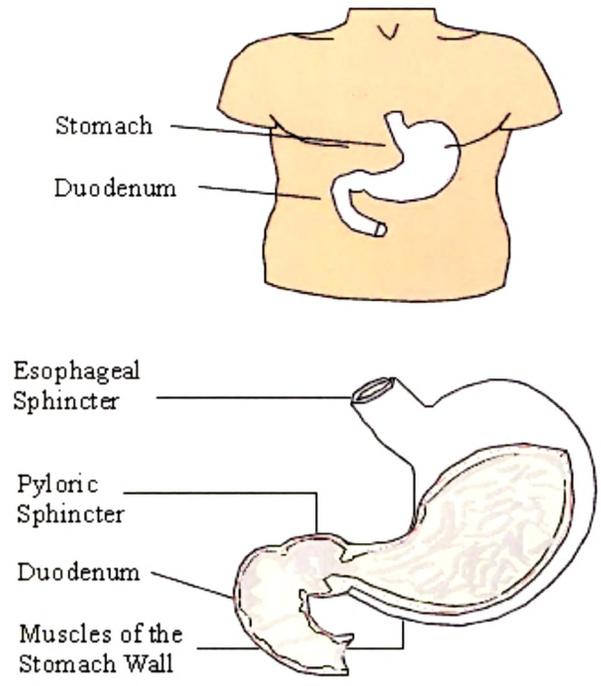


Figure 10: The Stomach and Duodenum

Without protection, the stomach's own hydrochloric acid and pepsin would eat away at the stomach wall. In order to protect itself, the stomach tissue employs a number of defenses and repair processes (Figure 11). The first line of defense is a layer of mucous, which contains bicarbonate to neutralize stomach acid. Next, cell membranes on the stomach wall contain lipids which repel water-soluble ions such as hydrogen. Finally, any ions that do penetrate the surface layer are removed by the underlying blood flow. The blood flow is also essential for developing the cap of mucus, and for maintaining the continually shed alkaline layer of fibrin and cellular debris beneath the mucous. In the absence of mucous, acid would begin to attack the first layer of stomach tissue. Normally, this would not be a problem because the first layer of stomach tissue is constantly being shed. But if the inner lining is destroyed faster than it can be replaced an ulcer may develop. With enough acidity and continued reach the underlying blood flow. When that happens, the ulcer is said to have perforated the stomach wall, allowing stomach acid and pepsin can directly act on the tissue underneath. At this point the person with the perforation would be

in considerable danger. If the acid reaches an artery, death may occur without prompt medical attention.

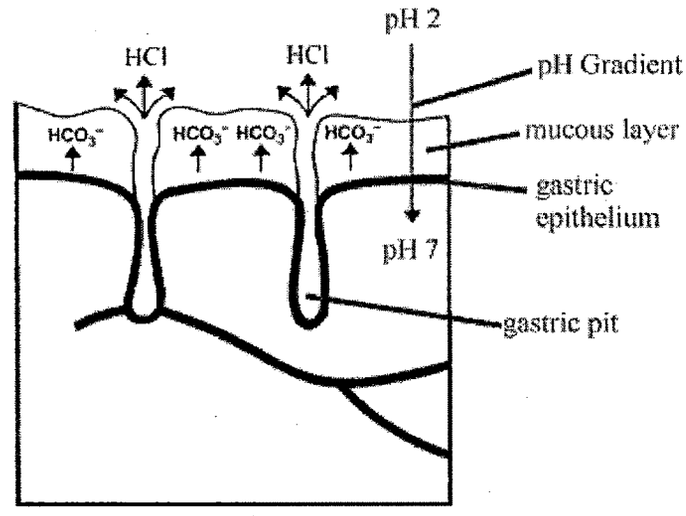


Figure 11: Stomach wall cross-section

1.23.2 Ulceration factors

a. NSAID uses

There are two components to NSAID-induced ulceration (Figure 12). First, there is a local acid effect of the dissolved drug. Most NSAIDs are weakly acidic, lipid-soluble compounds. Since the cell membranes on the stomach wall contain lipids for protection against strong acids, they offer little resistance to the lipid-soluble NSAID. The NSAID acts against the cell membrane, increasing its permeability. This results in cell swelling and death . The local acid effect of NSAIDs has been reduced by enteric-coating the drug, delaying dissolution until later in the digestive process. However, not all NSAIDs are enteric-coated as it increases cost. In addition, enteric-coating does little more than improve the symptoms of upset stomach. Patients must be informed that enteric-coated NSAIDs are still just as likely to cause stomach ulcers as regular NSAIDs.

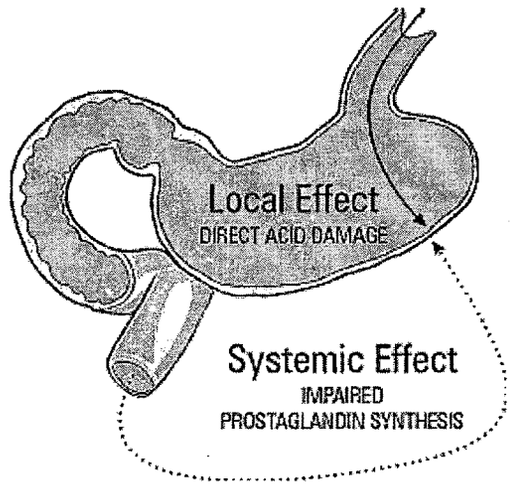


Figure 12: The Dual Insult of NSAIDs

The second and much more significant component to NSAID-induced ulceration is the systemic effect after being absorbed into the bloodstream. As described in the How NSAIDs Work, NSAIDs inhibit COX-1, reducing prostaglandin production. Normal COX-1 present in stomach tissue produces prostaglandins which:

- increase mucous and bicarbonate production,
- inhibit stomach acid secretion,
- increase blood flow within the stomach wall.

By acting on COX-1, NSAIDs restrict these self-protection mechanisms, allowing stomach ulcers to develop. It is primarily through this mechanism, not a local acid effect, that NSAIDs cause stomach ulcers. Unfortunately, the general public is not aware of this fact. Despite announcements by the U.S. Food and Drug Administration about the dangers of NSAIDs and calling for increased warnings on NSAID packaging.[20,21] patients are still receiving conflicting information. Bayer continues to produce advertising that promotes use of its coated aspirin tablets for people who experience stomach upset. Although Bayer's promotional literature is technically correct, it does not educate consumers about the risks associated with long-term aspirin use. This gives patients a false sense of security, and they are generally unaware that aspirin is one of the more dangerous NSAIDs. Aspirin in

particular was recently found to be a more common cause of GI perforation than previously recognized. [22] The responsibility for patient education about aspirin use lies with both physicians and pharmacists.

b. Helicobacter pylori infection

Complicating our understanding of stomach ulcers is Barry Marshall and Robin Warren's discovery in 1982 that bacteria are the primary cause of stomach and duodenal ulcers.[23] Helicobacter pylori is a spiral-shaped bacterium that lives in the stomach and duodenum, despite the highly acidic environment. It takes advantage of the stomach's own mucous for protection. Any acid that does reach the bacteria is converted by H. pylori's urease enzyme in the following reaction.[24]

urea + stomach acid + water --> bicarbonate + ammonia

$C{=}O{\cdot}2NH_2 + H^+ + 2H_2O \rightarrow HCO_3^- + 2NH_4^+$

The products of this reaction, bicarbonate and ammonia, are strong bases that further protect the bacteria because of their acid-neutralizing capability. The body's immune system responds to the presence of H. pylori and sends infection-fighting cells to the area. However, the neutrophils cannot reach the Helicobacter pylori infection because they cannot easily get through the stomach lining. Inflammation in the stomach tissue occurs as the neutrophils die and release super oxide radicals on the stomach wall, damaging tissue. The immune system sends in more nutrients to help the neutrophils, and the H. pylori can feed on these nutrients. It may not be the H. pylori itself that causes a stomach ulcer, but inflammation in the stomach lining as part of the immune response.[24]

It was also necessary to assume that without either NSAIDs or H. pylori, no ulcers of any type would form. After some other minor adjustments to the constraints, feasible solutions were obtained. A final solution is shown in Table 4.

Table 3: Assumptions Used In Ulceration Estimation Analysis

Constraints	Final Value
Risk of developing an ulcer while on an NSAID is 3 times higher than the total population risk.	8 585
Total number of ulcers is 1.5% of the population. (Concurrent gastric and duodenal ulceration counted as a single ulcer). *Source:*	15 000
Twice as many Duodenal ulcers as gastric ulcers. *Source: Calculated from*	4 977
Sum of all NSAID-related gastric ulcers is between 2% and 4%. *Source:* In the absence of additional risk factors, the chance of developing an NSAID ulcer is 2%. But with combinations of risk factors it can range up to 18%. It is the presence of these patients that results in an average of 2-4% incidence of ulcers in the population.	2.6%
Combined Duodenal & Gastric Ulceration is 0.07% of the population.	70
Sum of all NSAID takers is 6.7% of the population.	67 000
Combination ulcers (duodenal & gastric): NSAID & H. pylori-induced ulcers are 3 times more common than just NSAID-induced combination ulcers.	3
Twice as many NSAID-only gastric ulcers as NSAID-only duodenal ulcers.	671
30% of gastric ulcers test negative for H. pylori.	1493

Table 4: Estimated Ulceration Prevalence Per 1000000 People Per Year *Source: Non-linear optimization by author, based on various studies subject to the constraints listed in Table 3*

Scenario	Duodenal Ulcer	Gastric Ulcer	Both Types of Ulcers	Ulcer	TOTALS (people)
Neither NSAID user nor H. pylori infection	**0**	**0**	**0**	288	651 288
NSAID user only	**0**	**671**	**0**	035	51 706
Both NSAID user and H. pylori infection	**7 092**	**822**	**3**	77	15 294
H. pylori infection only	**2 862**	**3 484**	**67**	300	281 712
TOTALS (people)	9 953	4 977	70	000	**10,00, 000**

An interesting result of the optimization is the implication that NSAIDs only cause gastric ulcers, not duodenal ulcers. Currently, there is disagreement in published material as to whether or not this is true. The result also implies that a combination of NSAIDs and H. pylori is much more likely to cause an ulcer than H. pylori alone. This may or may not be true, and so requires further clinical research to confirm. Another implication is that NSAID use carries a much greater risk for gastrointestinal ulceration than H. pylori infection. This is borne out in real data which shows that while many ulcers occur as a result of H. pylori infection, serious gastrointestinal problems such as bleeding and perforation are much more strongly associated with NSAID use than H. pylori infection.[26] It is suggested that further study be done in an attempt to confirm the trends indicated in this non-linear optimization solution. Based on the experience gained in performing this optimization, future optimization attempts should concentrate on identifying the accuracy, precision and validity of the constraints and assumptions.

c. NSAID-associated ulceration risk factors

One aid to the physician in determining the possibility of NSAID-induced gastrointestinal damage in a particular patient is a set of established risk factors. Using the ARAMIS (Arthritis, Rheumatism and Aging Medical Information System) database in the USA, Fries has established the following simple model for estimating ulceration risk while taking NSAIDs:

1. Start at 0.
2. Add 0.3 for every 5 years over the age of 50.
3. Add 1.2 if the patient is receiving prednisone.
4. Add 1.4 if the patient has reported a previous NSAID-related GI side effect.
5. Add 0.5 if the patient has substantial disability.

The sum of these numbers represents the percentage risk of a patient experiencing a substantial adverse event in the next 12 months. Another study, the MUCOSA (Misoprostol Ulcer Complications Outcomes Safety Assessment) trial enrolled over 8800 patients with rheumatoid arthritis 52 years of age or older with at least 6 months of NSAID therapy. The trial identified four risk factors:

1. Age greater than 75 years
2. History of peptic ulcer
3. History of GI bleeding
4. History of heart disease

Combinations of the risk factors identified in the MUCOSA trial adds to increased risk of developing an ulcer, as illustrated in Figure 13.

Nonsteroidal anti-inflammatory drugs provide benefit by acting on the cyclooxygenase-2 enzyme. At the same time they can cause gastric ulcers by acting on cyclooxygenase-1 enzyme. Analgesics such as acetaminophen may be more specific to a third form of cyclooxygenase located primarily in the brain and responsible for fever and pain. Some NSAIDs have worse side effects than others, although they have the same anti-inflammatory action. This is due to differing specificity between COX-2 and COX-1 for each drug. NSAIDs do not usually cause ulcers by irritating the stomach. Instead, they cause ulcers by blocking the production of protective prostaglandins in the stomach. For this reason, an enteric-coated NSAID is less likely to cause stomach upset but just as likely to cause a stomach ulcer.

Traditional ulcer treatments have failed to prevent ulcers in NSAID users. One drug that does prevent NSAID-induced ulcers is misoprostol, a synthetic prostaglandin. Misoprostol is a useful drug with many possible applications besides ulcer prevention, but requires supervision against its misuse as an abortifacient. Upcoming developments include: 1) NSAIDs that contain nitric oxide to prevent ulcers and even heal existing lesions and 2) COX-2 specific NSAIDs that will no longer block the production of prostaglandins involved in gastric protection, reducing the possibility of causing an ulcer.

1.3 NO-NSAIDs

COX-inhibiting nitric oxide donators (CINODs), also known as **NO-NSAIDs**, are a new class of non-steroidal anti-inflammatory drug (NSAID) developed with the intention of providing greater safety than existing NSAIDs.[36] These compounds were first described by John Wallace and colleagues. CINODs are hybrid compounds generated by the fusion of an existing NSAID with a nitric oxide (NO)-donating moiety by chemical means, usually by ester linkage. CINODs retain the anti-inflammatory efficacy of NSAIDs *via* inhibition of cyclooxygenase (COX) while arguably improving upon gastric and vascular safety, most likely *via*

vasorelaxation, inhibition of leukocyte adhesion and reduction of apoptosis, all known effects of NO.

The first CINODs were developed in the 1990s, and as yet none have been approved for use by the general public. The importance of developing such drugs was increased when COX-2-specific NSAIDs rofecoxib (Vioxx) and lumiracoxib (Prexige) were removed from major pharmaceutical markets in the mid-2000s due to vascular safety concerns. Several CINODs are currently being tested in clinical trials, the most advanced of which are being conducted by the French pharmaceutical company NicOx, whose flagship compound naproxcinod is in phase III trials for the treatment of osteoarthritis.[2] Naproxcinod, also known as HCT 3012, AZD3582, NO-naproxen and nitronaproxen, is a fusion of naproxen and a NO-donating group. Other CINODs are also being tested by NicOx for the treatment of diseases in which inflammation plays a role.

1.4 Natural anti-inflammatory agents

Since ancient times our ancestors have used phytochemicals found in plants to curtail the inflammatory process. For example, the bark of the willow tree was used as an analgesic and antipyretic medication more than 2400 years ago by the Greeks and Romans.[37] The discovery of aspirin in 1899 was based on this observation.

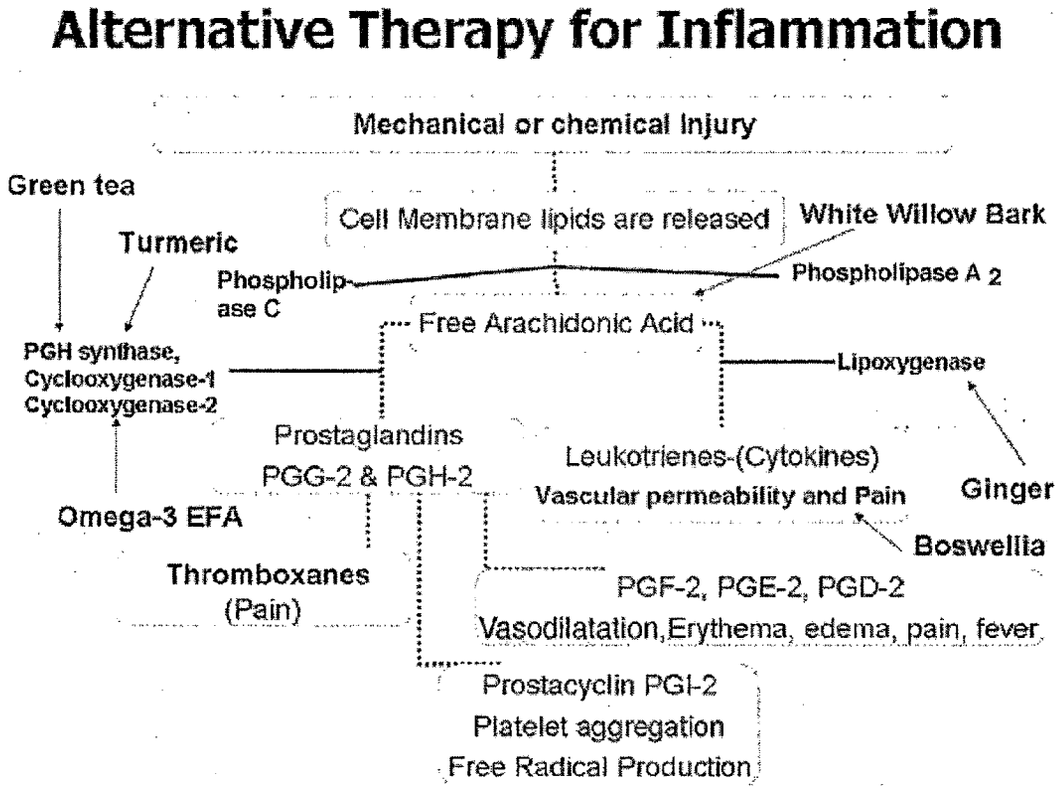


Figure 14. Schematic showing the inflammatory pathway, starting with tissue injury and ending with the inflammatory prostaglandins, thromboxanes, and leukotrienes. The natural anti-inflammatory agents omega-3 EFA, white willow bark, turmeric (curcumin), green tea, and *Boswellia* have all been shown to have various abilities to block certain parts of this pathway. Pycnogenol, cat's claw, and capsaicin also have anti-inflammatory effects associated with their action on the NF-κB pathway. PGD-2 = prostaglandin D2; PGG = prostaglandin G; PGH = prostaglandin H.

The emergence of today's pharmaceutical industry, in large part, has been based on natural products. Drugs such as digoxin, Taxol, artemisinin, and scores more have been developed from phytochemicals.[38,39] Not only have many medical breakthroughs been based on compounds of natural origin, but these also represent a large share of the drug market. In 1999, close to 50% of the 20 best-selling drugs were derived from natural products, and their sales amounted to approximately $16 billion.[38,40] According to a survey by the National Cancer Institute, 61% of the 877 small molecules, which are new chemical entities introduced as drugs worldwide from 1981 to 2002, were inspired by natural products. The following is a discussion of the most commonly used natural anti-inflammatory agents and their mechanism of action.

1.41 Omega-3 EFAs (fish oil)

The use of fish oil (in the form of cod liver oil), an omega- 3 EFA, for the treatment of muscular, skeletal, and discogenic diseases can be traced back to the late 18th century. As detailed by Curtis, et al.,[41] Dr. Thomas Percival recommended 1 to 3 tablespoons of cod liver oil two to four times per day for the treatment of "obstinate chronic rheumatisms, sciaticas of long standing, and in those cases of premature decrepitude, which originate from immoderate labor, repeated strains and bruises, or exposure to continuous dampness and cold; by which the muscles and tendons become too rigid, and the flexibility of the joints is impaired, so as to crackle for want of a due secretion of synovia." Unfortunately, because of the rapid onset of rancidity of this polyunsaturated oil when exposed to air and hence its disconcerting odor, cod liver oil fell out of favor.

With recently developed extraction techniques, which are performed under a nitrogen blanket, and with enhanced oxygen-free encapsulation methods, which prevent oxidation, the therapeutic benefits of fish oil can now be realized without the regurgitation and odor of previous products. Research has shown that the omega-3 polyunsaturated fatty acids are some of the most effective natural anti-inflammatory agents available.[41,42,43] With the discovery that vascular inflammation is the

underlying cause of coronary artery disease, fish and fish oil supplements are now recommended by the American Heart Association for the prevention of this disease.[44] Countries in which the highest fish consumption occurs have populations with a lower incidence of neurodegenerative disease and depression. The biological basis for the effectiveness of fish oil in treating arthritis has been well documented, with many positive clinical studies when compared with traditional pharmaceutical antiinflammaroty agents.

The active ingredients in fish oil, EPA and DHA, enhance the conversion of COX to prostaglandin E3. A natural antiinflammaroty agent, prostaglandin E3 competitively inhibits the effects of the arachidonic acid conversion to prostaglandin E2, a highly inflammatory substance. Prostaglandin E3 also inhibits the synthesis of TNFα and IL-1β, both of which are inflammatory cytokines. The EPA and DHA can inhibit the 5-LOX pathway, which converts arachidonic acid to inflammatory leukotrienes, also by competitive inhibition. When EPA and DHA are incorporated into articular cartridge chondrocyte cell membranes, there is a dose-dependent decrease in the expression and activity of the proteoglycan-degrading aggrecanase enzymes.

Omega-3 EFA, found in fish oil, can directly reduce the degenerative enzymes aggrecanase and matrix metalloproteinase, as well as IL-1, TNFα, and COX-2 to reduce the inflammation in synovial cartilage. A recent study of 250 patients with cervical and lumbar disc disease who were taking NSAIDs revealed that 59% could substitute fish oil supplements as a natural anti-inflammatory agent for the NSAIDs. The recommended dosage is a total of 1.5 to 5 g of EPA and DHA per day, taken with meals. Rare side effects include steatorrhea and occasional belching if the supplements are not taken with meals. Typically, persons on a regimen of anticoagulant medications should not take omega-3 EFAs because of the possibility of increasing the bleeding potential.

1.42 White willow bark [45]

Bark from the white willow tree is one of the oldest herbal remedies for pain and inflammation. It has been used by the ancient Egyptian, Roman, Greek, and Indian civilizations as an analgesic and antipyretic agent. In fact, the first record of its use is found in the Ebers papyrus, written more than 3500 years ago. Because the drug caused gastric irritation, the French chemist Charles Gerhardt neutralized salicylic acid and created acetylsalicylic acid. In 1897, Felix Hoffmann used the agent to treat his father's rheumatoid arthritis, and because of his success, the Bayer Corporation marketed the product under the trade name of aspirin, which is now one of the most widely prescribed nutraceutical agents in the world.

Because of the side effects of aspirin, there has been a resurgence in the use of white willow bark for the treatment of inflammatory syndromes. Salix alba, or white willow, is the species most commonly used for medicinal purposes. The mechanism of action of white willow bark is similar to that of aspirin in that it is also a nonselective inhibitor of COX-1 and COX-2, thus reducing the inflammatory prostaglandins. Various randomized placebo controlled studies comparing white willow bark with nonsteroidal agents have showed an efficacy comparable to these agents and aspirin. Salicin from white willow bark is converted to salicylic acid by the liver and is considered to have fewer side effects than aspirin. However, it is more costly than aspirin, and should not be used in children (to avoid the risk of Reye syndrome), or in patients with peptic ulcer disease, diabetes, hepatic or renal disorders, or other conditions in which aspirin would be contraindicated. The usual dose of white willow bark is 240 mg per day.

1.43 Curcumin (Turmeric)

Curcumin is a naturally occurring yellow pigment derived from turmeric *(Curcuma longa),* a flowering plant in the ginger family. It has traditionally been used as a coloring and flavoring spice in food products. Curcumin has long been used in both Ayurvedic and Chinese medicine as an anti-inflammatory agent, a treatment for

digestive disorders, and to enhance wound healing. Several clinical trials have demonstrated curcumin's antioxidant, anti-inflammatory, and antineoplastic effects.[46] In a recent article in the *New England Journal of Medicine*, Zandi and Karin suggested that curcumin might be efficacious in the treatment of cystic fibrosis because of its anti-inflammatory effect. Curcumin is known to inhibit inflammation by suppressing NF-κB, restricting various activators of NF-κB as well as stemming its expression. [47] Curcumin has been suggested as a treatment for colitis, chronic neurodegenerative diseases, arthritis, and cancer.[In addition, it regulates the activity of several enzymes and cytokines by inhibiting both COX-1 and-2. Most studies to date have been performed in animals, but given the centuries of use of curcumin, as well as its now demonstrated activity in the NF-κB, COX-1, and COX-2 inflammatory pathways, it may be considered a viable natural alternative to nonsteroidal agents for the treatment of inflammation.

The usual dosage of standardized turmeric powder is 400 to 600 mg taken three times per day. Side effects are few, but with extended use this agent can cause stomach upset, and in extreme cases gastric ulcers may occur at very high doses. Caution should be used if the patient is taking anticoagulant medications or high doses of nonsteroidal drugs. Studies have shown that Curcumin may be used in combination with lower doses of nonsteroidal medications.[48] Curcumin's therapeutic effects are considered comparable to pharmaceutical nonsteroidal medications such as phenylbutazone, but with a major difference in that this compound is relatively nontoxic and free of side effects.

1.44 Green tea

Green tea has long been recognized to have cardiovascular and cancer preventative characteristics due to its antioxidant properties.[49] Its use in the treatment of arthritic disease as an anti-inflammatory agent has been recognized more recently. The constituents of green tea are polyphenolic compounds called catechins, and epigallocatechin- 3 galate is the most abundant catechin in green tea.[50] Epigallocatechin-3 galate inhibits IL-1–induced proteoglycan release and Type

2 collagen degradation in cartilage explants.[51] In human *in vitro* models, it also suppresses IL-1β and attenuates activation of the transcription factor NF-κB. Green tea also inhibits the aggrecanases, which degrade cartilage.

From various studies, the molecular basis of the anti-inflammatory and chondroprotective effects of green tea is being discovered. A recent review article from Yale University regarding green tea as the Asian paradox summarizes its currently recognized therapeutic effects: as a cardiovascular and neuroprotective agent, an inhibitor of carcinogenesis, and an anti-inflammatory agent. The usual recommendation is 3 to 4 cups of tea a day. If the patient is taking green tea extract, a dosage of 300 to 400 mg is typical. Green tea can cause stomach irritation in some, and because of its high caffeine content, a decaffeinated variety should be considered

1.45 Pycnogenol (maritime pine bark)

Pycnogenol, like white willow bark, is a nutraceutical material that has been used since ancient times. Pycnogenol is derived from the bark of the maritime pine tree *(Pinus maritima)* and has been used for more than 2000 years.[52] Hippocrates mentions its use as an anti-inflammatory agent. It has been considered helpful for wound healing, treating scurvy, healing of ulcers, and reducing vascular inflammation. It contains a potent blend of active polyphenols that includes catechin, taxifolin, procyanidins, and phenolic acids. It is one of the most potent antioxidant compounds currently known. Pycnogenol inhibits TNFα-induced NF-κB activation as well as adhesion molecule expression in the endothelium.[123] Grimm and colleagues recently reported that oral intake of pycnogenol inhibited NF-κB activation in lipopolysaccharide- stimulated monocytes as well, thus decreasing the inflammatory response. It also statistically significantly inhibited matrix metalloproteinase–9.[53] This matrix-degrading enzyme is highly expressed at sites of inflammation, and contributes to the pathogenesis of various chronic inflammatory diseases.

In a recently published review article on pycnogenol and its effect on the cardiovascular system, investigators concluded that due to its anti-inflammatory activity, this agent has the potential to counteract major cardiovascular risk factors, including reducing platelet activity and reducing the inflammatory process that underlies coronary artery disease. With the mounting evidence of its anti-inflammatory effects and its virtual absence of toxicity, pycnogenol may play a larger role in the treatment of the pain from arthritic conditions in athletes as well as in degenerative disease of all kinds. Due to its potent antioxidant effects, enhancement of sports endurance was indicated in a recent study in which athletes took 200 mg per day of pycnogenol. Vigorous sports activity dramatically increases oxygen consumption, by 10- to 20-fold over the resting state. Hence, an increased number of free radicals is generated during exhaustive exercise. Pycnogenol is thought to counteract the deleterious effects of these free radicals and improve blood flow to muscle, as was demonstrated by Pavlovic in a double-blind cross-over study of 24 recreational athletes.

Studies have shown that this agent is 50 to 100 times more potent than vitamin E in neutralizing free radicals and that it helps recycle and prolong the activity of vitamins C and E. Studies have shown pycnogenol to be effective in reducing blood pressure and reducing the risk of venous thrombosis by its effect on vascular endothelium. The usual dosage is 100 to 200 mg daily. Few side effects from the use of pine bark extracts have been reported, the most frequent being mild gastrointestinal effects such as diarrhea and upset stomach.[102,103] Pycnogenol should not be taken by patients who are being treated with immunosuppressants or by those receiving corticosteroid drugs, because it can enhance immune system function and interact with drugs that are supposed to suppress the immune system.[53]

1.46 Boswellia serrata resin (Frankincense)[54]

The *Boswellia* species are trees located in India, Ethiopia, Somalia, and the Arabian peninsula that produce a gum resin called olibanum, better known in the

western world as frankincense. This resin possesses anti-inflammatory, antiarthritic, and analgesic properties. It is known to inhibit the leukotriene biosynthesis in neutrophilic granulocytes by inhibiting 5-LOX. Various inflammatory diseases are perpetuated by leukotrienes, hence some of the anti-inflammatory activity of this agent. Clinically, the substance is used in the treatment of degenerative and inflammatory joint disorders. It reduces the total white blood cell count in joint fluid, and it also inhibits leukocyte elastase, which is released in rheumatoid arthritis. In one recent study, a statistically significant improvement in arthritis of the knee was shown after 8 weeks of treatment with 333 mg *B. serrata* extract taken three times a day. The treatment improved function, but radiographically there was no change in the affected joints. Another study by Kulkarni, et al[55], demonstrated a significant drop in pain severity and disability. A combination of *Boswellia* and curcumin showed superior efficacy and tolerability compared with nonsteroidal diclofenac for treating active osteoarthritis. *Boswellia* typically is given as an extract standardized to contain 30 to 40% boswellic acids (300– 500 mg two or three times/day). *Boswellia* has been well tolerated in most studies, although some people may experience stomach discomfort, including nausea, acid reflux, or diarrhea.

1.47 Uncaria tomentosa *(Cat's claw)*

Uncaria tomentosa [56] is Peruvian herbs derived from woody vines with small clawlike thorns (hence the vernacular name, cat's claw) at the base of the leaf that allows the plant to climb to heights of up to 100 ft. Traditionally, a decoction of the bark of the cat's claw is used to treat arthritis, bursitis, and intestinal disorders.[57] The active ingredients appear to be polyphenols (flavonoids, proanthocyanidins, an tannins), alkaloids, and sterols. Various studies indicate that this Peruvian herb induces a generalized reduction in proinflammatory mediators.[58]

This herb has been shown to prevent the activation of the transcriptional factor NF-κB and it directly inhibits TNFα production by up to 65 to 85%. It inhibits the expression of inducible genes associated with inflammation, specifically negating the expression of inducible nitric oxide synthetase, and hence attenuates nitrous

oxide production. Side effects may include nausea, although it has shown an impressive protective effect on indomethacin- induced enteritis in laboratory studies. Although toxicity and side effects are usually minimal, two case reports of acute renal failure in a patient with lupus erythematosus have been recorded. Cat's claw can be consumed as a tea (1000 mg root bark to 8 oz water), or as a dry, standardized extract in a capsule (20–60 mg daily).

1.48 Capsaicin (Chili pepper)

Capsicum annum is a small spreading shrub originally cultivated in the tropical regions of the Americas but now is grown throughout the world, including the US[59]. The small red fruit commonly used to accentuate chili owes its stinging pungency to the chemical capsaicin. This was isolated by chemists more than a century ago and constitutes approximately 12% of the chili pepper. This fruit has been used for medicinal purposes by the native peoples of the American tropics for hundreds of years.

More recently, various preparations have become available over the counter for the treatment of peripheral neuropathies and chronic musculoskeletal pain. Capsaicin produces highly selective regional anesthesia by causing degeneration of capsaicin-sensitive nociceptive nerve endings, which can produce significant and long-lasting increases in nociceptive thresholds. Capsaicin potently activates transient receptor potential vanilloid 1, which is a main receptor underlying nociception.[60] It also inhibits NF-κB, thus producing an antiinflammaroty effect. Capsaicin can cause a burning sensation when it comes in contact with human flesh, and also in the digestive tract. This herb is rarely used alone but is generally mixed into other natural antiarthritic preparations.[60] There are topical capsaicin formulations now available to treat post therapeutic neuralgia.