

# Inflammation and Hyaluronic Acid

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**H**yaluronic acid (HA) has been known for more than 70 years, but the focus has been on its physical properties—as a replacement for the vitreous humor of the eye, as a lubricant in the joints, or as a humectant in high-end cosmetics. More recently, HA has been recognized as having anti-inflammatory properties, and the molecular weight of the molecule has been established as an important feature of the molecule. HAs of higher molecular weight (HMW) tend to be anti-inflammatory, whereas those of lower molecular weight (LMW) tend to be proinflammatory. It has also been found that LMW fragments of HA, as well as the proinflammatory molecules they generate, stimulate the genes that encode HA synthase, thereby yielding more HA with a preference for the HMW form of this essential molecule.<sup>1–3</sup> Overwhelming evidence now suggests that HA functions as a biologically endogenous anti-inflammatory molecule, resolving both acute and chronic inflammation *in vivo*.<sup>4</sup>

## Structure of HA

HA is a naturally occurring biopolymer belonging to a class of compounds known as glycosaminoglycans (GAGs). In addition to HA, GAGs also include chondroitin sulfate, dermatin sulfate, keratin sulfate, heparin, and heparan sulfate. HA is the only GAG that is nonsulfated. Molecules of HA consist of repeating units of N-acetylglucosamine and glucuronic acid, and these polymers can range in size from 5000 to 20,000,000 Daltons (Da).

## Causes of Pain and Swelling

Inflammation is a defensive reaction of the body in response to harmful stimuli, such as pathogens, damaged cells, or irritants. Two of the primary characteristics of inflammation are pain and swelling. In the biologic process known as the leukocyte adhesion cascade (see Fig. 1), the leukocytes are directed to the site of trauma in order to allow for repair of damage to tissue.<sup>5</sup> With trauma, proinflammatory cytokines are produced, the most important of which are interleukin-1 (IL-1), interleukin-6 (IL-6), tumor necrosis factor (TNF),<sup>6</sup> and interferon.<sup>7</sup> These cytokines activate the endothelium of the blood vessels in the affected area. The endothelium expresses cellular adhesion molecules (CAMs) that recruit circulating leukocytes to the surface of the endothelium, where they “roll” to the site of injury. This rolling is accomplished by the successive attachment and release of the leukocyte to the endothelium via cell surface receptors.

When the leukocytes reach the site of injury, they secrete a small amount of protease, which breaks down the blood-vessel wall and allows the leukocyte to move into the surrounding tissue. Here the leukocytes phagocytize damaged cells (i.e., engulf the foreign substance, degrade it, and finally expel it from the cell) and allow the tissue to repair. Often, however, the body overreacts, sending more leukocytes than needed to clean up the tissue.

Swelling is also caused by increased vascular permeability promoted by inflammatory molecules which leads to edema in the affected area.<sup>8,9</sup> Edema, along with the buildup of leukocytes in the tissue, contributes to swelling. The associated pain is caused by compression of nociceptors, in the affected area, as well as nerve pain caused by the inflammatory molecules generated by the trauma itself.

## How HA Reduces Swelling

Some time ago, Dermal Research Laboratories Inc. (Kansas City, Missouri), had noticed that its patented topical liquid formulation of HA had a profound effect on pain and swelling. Immediately upon application of the product to the site of trauma, pain was relieved and the swelling could be seen to diminish within a few minutes after application. The effect was quite dramatic. This prompted a search for reasons behind these observations.

HA is known to slow the movement of leukocytes<sup>10,11</sup> and to make an impact on neutrophil adhesion within blood vessels.<sup>12,13</sup> Dermal scientists reasoned that if *excess* HA is present in the bloodstream, this HA interferes with the movement of leukocytes by binding to its ligand, CD44. CD44 is a glycoprotein that resides on the surfaces of leukocytes, as well as on the endothelial linings of the blood vessels. This glycoprotein is responsible for various cell–cell interactions and specifically binds HA. If HA blocks the binding sites for CD44 on the surface of the leukocyte and on the endothelium, the “rolling” action of the leukocyte is diminished. As a result, fewer white blood cells migrate to the site of inflammation and then into the surrounding tissue, reducing the degree of swelling in the area of injury (see Fig. 2).

It is important to note that, although HA slows the leukocyte “rolling” process, HA does not totally prevent it. For this reason, sufficient white blood cells still reach the site of trauma to provide healing, but excessive swelling and pain are reduced.

HA also reduces the edema associated with the pain and swelling. It has been found that HMW HA reduces vascular permeability both *in vitro* and *in vivo*.<sup>14,15</sup>

## Role of PG Pathway in health

PGs, prostacyclins, thromboxanes, leukotrienes, and related compounds are examples of eicosanoids. Eicosanoids carry signals that affect their target cells locally, but are rapidly degraded, and not transported to other sites within the body. Under normal physiologic conditions, these eicosanoids are released as needed to keep the organism in homeostasis. As shown in Figure 3, arachidonic acid (AA), the beginning of the PG pathway, is in equilibrium with the phospholipid membrane. Healthy tissues create just enough of the individual prostanoids (PGE<sub>2</sub>, PGI<sub>2</sub>, PGD<sub>2</sub>, thromboxane, etc.) to promote proper functioning. As can be seen, these small molecules often work in opposition to one another, and an individual prostanoid can even function differently in different tissues. In normal healthy tissues, this pathway responds, as necessary, to make the appropriate eicosanoid to keep the organism in balance chemically.

## Primary Mechanism for Pain Prevention

Imbalance in the PG pathway is the primary mechanism for pain production. When trauma occurs, proinflammatory PGs are upregulated through increased production of AA, resulting in pain and inflammation. As seen in Figure 4, a traumatic event, such as tissue damage or the introduction of a foreign substance, releases a variety of molecules that initiate the events that eventually lead to pain. The interleukins, IL-1 and IL-6, and TNF, for example, upregulate phospholipase A (PLA)-2, the enzyme catalyzing the conversion of membrane phospholipids into AA. Likewise, biogenic amines, such as histamines, are released into the tissues by immune cells and also increase AA production.<sup>16</sup>

These initiators of the inflammatory process also promote the conversion of kininogen to bradykinin via the enzyme kallikrein. Bradykinin, in turn, also shifts the equilibrium toward AA production, but has nociceptive activity itself, thus producing pain independent of the prostanoids. The result is a strong shift in the equilibrium toward increased production of AA.

Cyclo-oxygenase (COX)-1 enzyme is constitutive in platelets, the gastric epithelial cells, central nervous system (CNS), vascular endothelial cells, and renal tubules. The COX-2 enzyme is constitutive in the kidney, brain, pancreas, intestines, reproductive tissues, thymus, spinal cord, and blood vessels.<sup>17</sup> The increased level of AA induces increased levels of COX-2 with an emphasis on PGE<sub>2</sub> and PGI<sub>2</sub>, proinflammatory prostaglandins.<sup>18-20</sup> The presence of these eicosanoids in the tissues stimulates neurons, creating pain.<sup>21</sup>

## Unwanted of Current Pain Relievers

An inflammatory environment results in the loss of equilibrium in the PG pathway. This upregulates proinflammatory prostanoids and results in pain.

The search for more effective and safer pain relief has been ongoing for decades, with particular focus on the PG pathway.<sup>22,23</sup> Figure 5 illustrates the anti-inflammatory activity of several classes of commercially available pain relievers as they relate to the PG

pathway.

Corticosteroids, known since the 1950s, are very effective pain relievers. This class of compounds blocks the activity of proinflammatory molecules *prior* to the initiation of the PG pathway. The compounds block the activity of IL-1,<sup>24</sup> IL-6,<sup>25</sup> and TNF<sup>26</sup> before these activators of the PG pathway can initiate the inflammation process (see Position 5 in Figure 5). In addition, corticosteroids block the activity of PLA-2<sup>27</sup> and prevent the formation of excess AA (see Position 1 in Figure 5). As a result, the balance within the PG pathway is not disrupted by use of corticosteroids. However, many other side effects of corticosteroids are well known,<sup>27, 28</sup> and for some time, corticosteroids have *not* generally been the pain reliever of choice.

Antihistamines, a second class of analgesics, prevent the release of histamine and thus block the pathway reaction at Position 2 (see Figure 5). However, antihistamine efficacy is spotty and side effects, such as drowsiness, are numerous.<sup>29-31</sup>

By far, the most common pain relievers sold today are nonsteroidal anti-inflammatory drugs (NSAIDs), which block the prostaglandin pathway at Position 3 (see Figure 5). This class of anti-inflammatories, which includes aspirin, rofecoxib (Vioxx<sup>®</sup>), and celecoxib (Celebrex<sup>®</sup>), are the most researched group of current and potential pain relievers. They block the conversion of AA to the prostanoids by inhibiting the COX-1 and COX-2 enzymes. COX inhibitors generally show a preference for blocking either the COX-1 pathway or the COX-2 pathway.

COX-1 inhibitors are the original NSAIDs. However, the side effects are numerous because, in addition to blocking the proinflammatory PGs, they also block the “good work” the PGs do, such as gastric protection and maintaining proper blood clotting. Recently, COX-2 products, such as Vioxx and Celebrex, block not only the proinflammatory PGs, but also block the prostanoids that stimulate vasodilation and reduce blood clotting. As a result, these products have now been associated with increased incidences of heart attack and stroke.<sup>32,33</sup>

5-Lipoxygenase (5-LOX) inhibitors are a new class of pain relievers that block the formation of leukotrienes. These pain relievers are associated with proper maintenance of the airways and are most commonly used for asthma and allergies.<sup>34-36</sup>

The basic problem with the use of NSAIDs is that, while blocking the proinflammatory effects of the PG pathway (i.e., pain), they also block the pathway’s protective, positive effects. As a result, the unintended consequences are a vast array of negative side effects, such as cardiovascular events, kidney failure, stomach bleeding, and stroke. Because of the risks associated with interference with the PG cascade, research on better pain relievers currently focuses on areas outside of the PG cascade.<sup>22,23</sup>

## Blocking Inflammation Without Disrupting the PG Pathway

HA inhibits the proinflammatory molecules of the PG pathway by blocking the initiators of inflammation.

Dermal Research Laboratories believes that the success of its HA formulations in blocking pain is a result of how the molecule interacts with the PG pathway. HA is a single molecular type that blocks all the

major proinflammatory stimuli leading to upregulation of the PG pathway, but does not block the internal balance within the pathway.

This is illustrated in Figure 6. HA's anti-inflammatory activity occurs by blocking the noxious inputs to the pathway.

When trauma occurs, HA blocks the reactions at Position 5 (shown in Fig. 6) that initiate the inflammatory response to trauma. HA blocks IL-1,<sup>37,38</sup> IL-6,<sup>39</sup> and TNF.<sup>40–42</sup> HA also blocks excess production of phospholipase (PLA-2),<sup>43</sup> thus not disrupting the balance of the prostaglandin pathway. In this respect, HA acts similarly to corticosteroids, but without the negative side effects. Intra-articular injections of HA appear to be as effective as the synthetic corticosteroids, methylprednisolone and triamcinolone, in treating osteoarthritis, and provide more sustained relief and better tolerability.<sup>44</sup> Similarly, injections of HA into the arthritic temporomandibular joint for pain relief proved superior to corticosteroids.<sup>45</sup>

HA blocks the conversion of kininogen to bradykinin at Position 3 (see Fig. 5),<sup>46</sup> by binding kallikrein, thus stopping the proinflammatory effects of bradykinin, including the stimulation of pain receptors. HA also blocks histamine production (see Position 2, Fig. 6).<sup>47</sup>

The result is that no excess AA is produced and the equilibrium within the prostaglandin pathway is preserved. Upregulation of the prostaglandin pathway does *not* occur, and pain and inflammation are blocked. Most importantly, however, is that HA does all of this without any known side effects.

## Inflammation and Aging

The presence of proinflammatory molecules increases with age. These molecules include IL-1, IL-6, IL-18, C-reactive protein (CRP), and TNF.<sup>48–49</sup> It has been suggested that this may be caused by impairment of the mechanisms that turn off the inflammatory response.<sup>50</sup> Inflammation has been linked to many of the diseases of aging, including osteoarthritis (OA), osteoporosis, insulin resistance, glaucoma, dementia, Alzheimer's disease, heart disease, stroke, multiple myeloma, atherosclerosis, asthma, macular degeneration, and sarcopenia (frailty).<sup>48,51–57</sup> A growing body of literature also links inflammation with cancer.<sup>58</sup>

## HA and Aging

Changes in the body's concentration of HA have long been of interest—particularly those changes that accompany aging. Downregulation of HA synthase is thought to contribute to joint disease, particularly OA, in dogs.<sup>59</sup>

It is well established that the viscoelastic properties of joint fluid decrease with age.<sup>60</sup> In human cartilage, the total HA content was found to increase substantially between the ages of 2.5 and 86 years, whereas the average molecular weight (MW) decreased from  $2.0 \times 10^6$  to  $0.3 \times 10^6$  Daltons. The same study also showed that newly synthesized HA had very high MW, regardless of the age of the cartilage, implying that aging was accompanied by modifications in the MW in the tissues over time with age.<sup>61</sup> This is consistent with the increasingly proinflammatory environment of tissues with aging, as noted above.

A decrease in the presence of HA in eye tissue after the fifth

decade is thought to contribute to age-related retinal disorders.<sup>62</sup> Individuals with glaucoma who ranged from 76 to 89 years of age, were found to have virtually no HA in areas of the optic nerve, while similar changes were not found in either age-matched persons with normal sight or in younger individuals.<sup>63</sup> Other studies show that HA depletion and an accumulation of chondroitin sulfate in the eyes may increase resistance to outflow of ocular fluid from the eyeball into general circulation, increasing intraocular pressure and causing glaucoma.<sup>64</sup>

The total amount, as well as the MW, of HA in the skin appears to remain constant with aging. However, HA becomes more tightly tissue-associated with advancing age. In a fetus, for example, 7% of the HA is tissue-associated, whereas the amount increases to 23% in senescent skin. Additionally, this HA accumulates in deeper layers of skin over time. With increasing age, HA is entirely lost from the epidermis, resulting in the dryness associated with aged skin.<sup>65</sup> This creates problems in wound healing and other inflammatory diseases involving the skin.<sup>66</sup>

## Conclusions

HA is known to block both acute and chronic inflammation.<sup>4</sup> HA diminishes pain and swelling by mitigating the adhesion cascade and reducing vascular permeability. HA is also known to block AA release in inflamed joints.<sup>67</sup> By blocking the initiators of inflammation, HA maintains equilibrium in the PG pathway, inhibits production of proinflammatory molecules, and this reduces pain and inflammation while avoiding unwanted side effects. A decrease in HA may contribute to the inflammatory diseases and conditions associated with aging. Table 1 summarizes the proinflammatory activities and the relationship of each of these activities to HA. □

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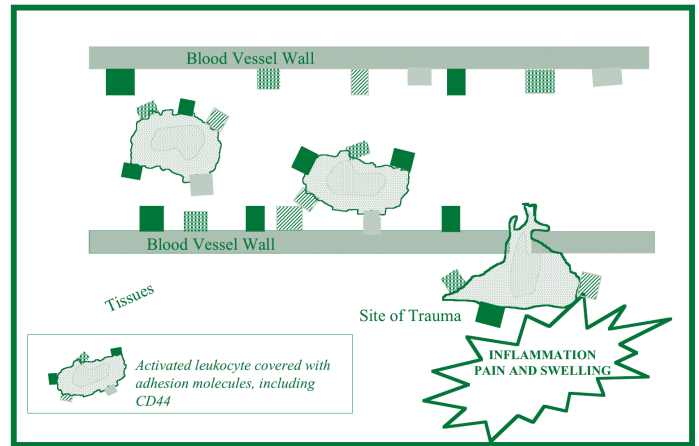
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**Figure 1. Leukocyte adhesion cascade.** The leukocyte adhesion cascade is the biologic process that directs white blood cells to the site of trauma. Inflammatory molecules generated by the event cause the vascular endothelium to attract white blood cells to the surface. The cells “roll” along the endothelium until they reach the site of the trauma. Here, the cells squeeze through a pore created in the vessel wall via extravasation and enter the tissue. The function of the leukocyte is to phagocytize the foreign substances and allow for tissue repair. Swelling and pain arise when the body overreacts and too many leukocytes arrive at the site. Used with permission of ©Dermal Research Laboratories Inc., Kansas City, Missouri.

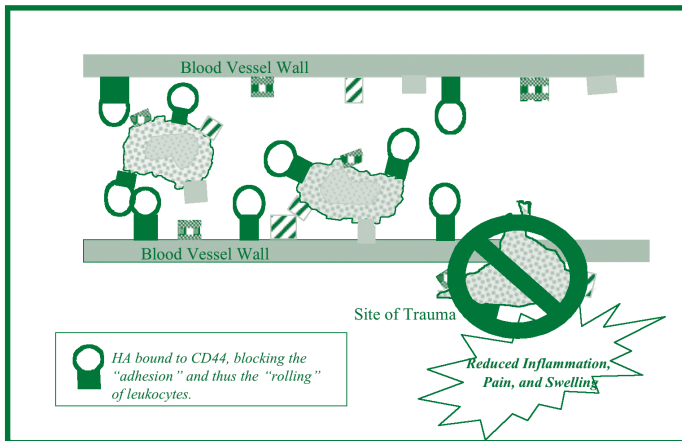


Figure 2. Adhesion cascade with excess hyaluronic acid (HA). In the presence of added HA, the activity of the leukocyte adhesion cascade is diminished. HA binds specifically to its ligand CD44, which resides on the vascular endothelium as well as on the surface of the white blood cells. This inhibits binding of the white cells and interrupts the rolling process. As a result, fewer white blood cells reach the site of trauma, and there is less swelling and pain. Used with permission of ©Dermal Research Laboratories, Inc., Kansas City, Missouri.

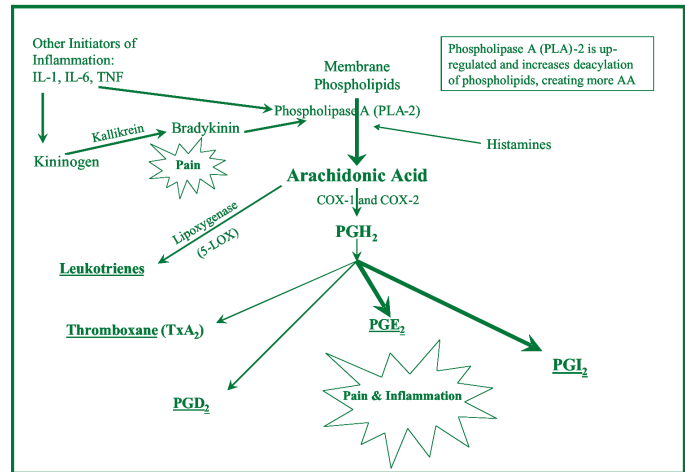


Figure 4. Prostaglandin (PG) pathway when trauma occurs. Trauma releases inflammatory molecules, such as interleukin (IL)-1, IL-6, and tumor necrosis factor (TNF) into the tissues. These upregulate phospholipase (PLA)-2 to produce more arachidonic acid (AA) via increased deacylation of the phospholipids in the cellular membranes. At the same time kininogen is increased, producing bradykinin, which also increases the activity of PLA. Histamines also increased the amount of AA in the tissues. As a consequence of increase amounts of AA the cyclooxygenase (COX)-2 enzyme is upregulated and produces proinflammatory PGE<sub>2</sub> and PGI<sub>2</sub> preferentially. Used with permission of ©Dermal Research Laboratories, Inc., Kansas City, Missouri.

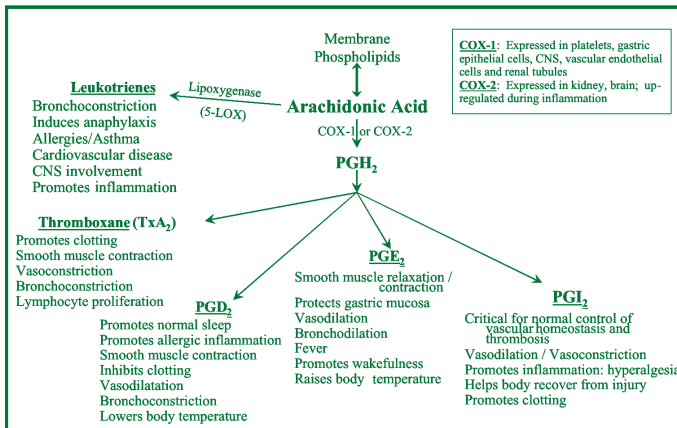


Figure 3. Prostaglandin (PG) pathway. PGs and related compounds are small cell-signaling molecules designed to keep the body in homeostasis. They are synthesized, as needed, from arachidonic acid. Their functions are diverse and often work in opposition to one another, as is the case with PGD<sub>2</sub>, which promotes normal sleep and PGE<sub>2</sub>, which promotes wakefulness. In some cases PGs can have opposite effects in different tissues. COX, cyclo-oxygenase; LOX, lipoxygenase; CNS, central nervous system. Used with permission of ©Dermal Research Laboratories, Inc., Kansas City, Missouri.

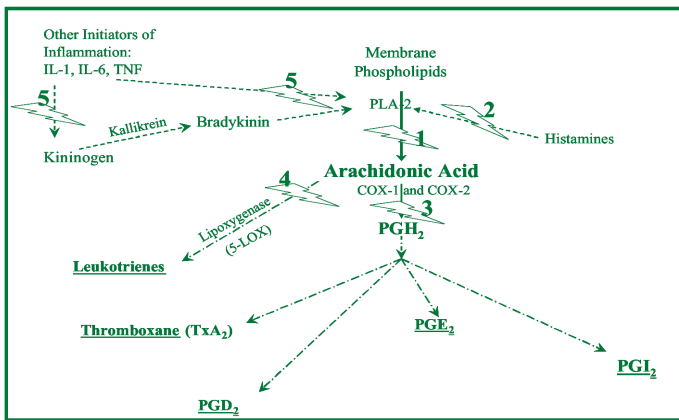


Figure 5. Prostaglandin pathway: with conventional pain relievers. Conventional pain relievers block the prostaglandin (PG) pathway at the points indicated by the numbers in this figure. Corticosteroids are very efficient pain relievers and block the pathway at Position 1 by inhibiting the phospholipase A (PLA)–2 and at Position 5 by blocking the major initiators of inflammation, including interleukin (IL)–1, IL-6, and tumor necrosis factor (TNF). Antihistamines block the pathway at Position 2 by blocking PLA-2. Nonsteroidal anti-inflammatory drugs (NSAIDs) block the pathway at Position 3. By blocking the conversion of arachidonic acid (AA) to prostanooids, the protective behaviors of these molecules are lost as well as their proinflammatory effects. A new class of pain relievers block leukotrienes at Position 4 by inhibiting the enzyme 5-lipoxygenase (5-LOX). COX, cyclo-oxygenase. Used with permission of ©Dermal Research Laboratories, Inc., Kansas City, Missouri.

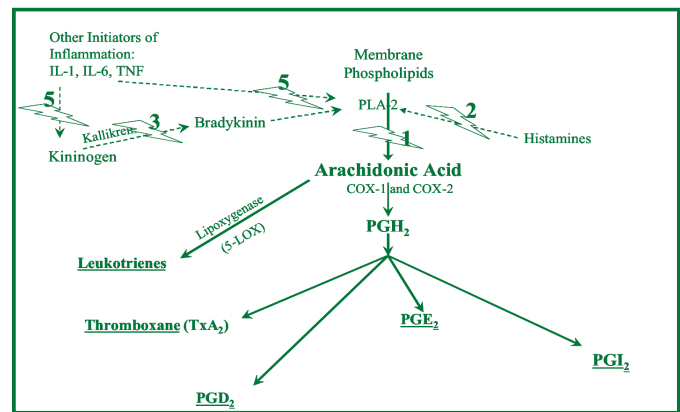


Figure 6. Prostaglandin (PG) pathway in the presence of hyaluronic acid (HA). In the presence of HA, the PG pathway continues to function in balance because the inhibition of inflammation, and thus pain, occurs prior to the formation of increased amounts of arachidonic acid (AA). Like corticosteroids, HA blocks the proinflammatory response at Positions 1 and 5. HA also blocks the activity of histamine and the activity of kallikrein to block formation of bradykinin. Used with permission of ©Dermal Research Laboratories, Inc., Kansas City, Missouri.

**Table 1. Inflammation and Hyaluronic Acid (HA)**

What happens during inflammation	Impact of HA and references
Acute and chronic inflammation	HA modulates both acute and chronic inflammation <i>in vivo</i> <sup>4</sup>
Increase in advanced glycation endproducts (AGEs), which are associated with amyloid plaque	HA inhibits inflammation associated with AGEs <sup>42</sup>
Increase in nuclear factor-kappa beta (NF- $\beta$ )	HA inhibits NF- $\beta$ <sup>42</sup>
Increased sensitivity of nociceptors	HA reduces nociceptive activity <sup>a</sup>
Increase in tumor necrosis factor (TNF)	HA inhibits TNF <sup>40–42</sup>
Increased activity of phospholipase, causing increases in AA	HA inhibits phospholipase, preventing formation of AA <sup>43</sup>
Increase in interleukin (IL)-1, an inflammatory cytokine	HA downregulates IL-1 <sup>37,38</sup>
Increase in IL-4, an inflammatory cytokine	HA inhibits IL-4 <sup>40</sup>
Increase in IL-6, an inflammatory cytokine	HA inhibits IL-6 <sup>39</sup>
Increase in edema and neutrophil infiltration	Decreases edema and neutrophil infiltration <sup>10,14,15</sup>
Increase in macrophage inflammatory protein (MIP-2)	HA inhibits MIP-2 <sup>40</sup>
Increase in bradykinin	HA inhibits formation of bradykinin <sup>46</sup>
Increase in AA production	HA inhibits formation of excess AA <sup>43</sup>
Increased histamine production as a result of activation of the innate immune system	HA inhibits histamine production <sup>47</sup>

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Superscripted numbers represent references in the text.

<sup>a</sup>Pozo MA, Balazs EA, Belmonte C. Reduction of sensory responses to passive movements of inflamed knee joint by hylan, a hyaluronic acid derivative. *Exp Brain Res* 1997;116:3–9.