REVIEW ARTICLE

Kartogenin - a potential small molecule for managing osteoarthritis

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Abstract

There is a current need to develop novel therapeutic strategies to improve osteoarthritis (OA) by reducing joint pain, slowing cartilage degeneration progress, and supporting cartilage regeneration. In order to reduce the progression of OA, several small molecules with chondrogenic, anti-inflammatory, and extracellular matrix modulatory activities are being investigated as potential OA medications. Kartogenin (KGN) is one such molecule recently identified through an image-based-high-throughput screening. Since its discovery, many studies have been conducted to determine its biological effects in chondrogenesis, increased cartilage regeneration, reduced OA-induced pain, reduced inflammation and role in modulating limb development. Additionally, many biomaterial-based carriers have been developed for the sustained release of KGN. Lastly, several biological mechanisms of action of KGN are identified too. These include the Filamin A/CBFβ/RUNX1, Indian Hedgehog (Ihh), TGFβ/Smad Pathway for Chondrogenesis, the AKT/PI3K and TAOK1/Hippo Pathways for its anti-inflammatory effects and IL-10 mediated analgesic effect. The observed impacts on the formation of cartilage cells, maintaining a balance of anabolic and catabolic effects, decreasing inflammation and offering pain alleviation, emphasise the potential benefits of administering KGN for treating osteoarthritis.

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Introduction

Osteoarthritis is a chronic joint disease characterized by degenerative changes in the joint tissues [1,2]. It is considered the most common form of arthritis affecting over 32.5 million adults in the United States alone and is a leading cause of disability among older adults [3]. Osteoarthritis results from injury to articular cartilage due to trauma, pathological disease, or wear and tear [4]. A hallmark of osteoarthritis is the progressive destruction of the articular cartilage extracellular matrix [5]. As articular cartilage has poor vascular supply, lymphatic drainage, and poor progenitor cell access to the lesions they lack the ability to regenerate upon injury. In addition, several risk factors have been identified that affect the development of osteoarthritis, including age, gender and weight. Even though osteoarthritis can affect any joint in the body, it commonly affects the hands, knees, hips, and spine. The major symptoms of osteoarthritis are joint pain and a decreased range of motion secondary to the pain. Other associated symptoms include stiffness and swelling.

These symptoms may be mild at first but can become more severe over time and as such, treatment options are increasingly important to improve the quality of life [4,6].

The current therapeutic options for osteoarthritis to reduce pain and functional disability includes non-pharmacological interventions to dietary supplements, pharmacological therapies, intra-articular injections and finally, invasive surgical approaches [7,8]. Exercise and physical therapy can help improve flexibility and strength of the affected joint and also help to reduce the strain on the joints. Topical or oral non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used to treat the pain and inflammation of osteoarthritis; however, the cardiovascular, gastrointestinal and renal side effects of NSAIDs raise significant concerns. Opioids which present another option, however, are limited by severe side effects, including addiction. Recently, targeted approaches to OA treatment with small molecules over palliative drugs are gaining interest. These include the local administration of small molecular drugs via intraarticular injections. The intraarticular injection of corticosteroids has shown an effective, short-term reduction in OA pain [2]. Similarly, capsaicin, a small molecule that binds to TRPV1 receptors, has been shown to provide OA pain relief via the desensitization of nerve fibers [9]. The injection of hyaluronic acid, also known as visco-supplementation, works as a short-term treatment for OA pain and improves joint functions by acting as a lubricant [10]. Lorecivivint is a potential disease-modifying osteoarthritis drug recently shown to improve OA joint pain and improve joint functions through its interaction with the Wnt signaling pathway [9,11]. These small molecules, however, do not provide an effective long-term solution to combat OA. Novel and or alternative therapeutic strategies are being investigated to improve the outcome by reducing joint pain, slowing down the progress of cartilage degeneration and supporting cartilage regeneration. Several small molecules with chondrogenic, anti-inflammatory and extracellular matrix modulatory properties are being investigated as disease modifying OA drugs to alleviate or even reverse the development of OA. Cell-based screening of small molecule libraries has led to the identification of several candidate molecules capable of supporting cartilage differentiation and protecting cartilage from degeneration. The review presents an overview of the recent studies demonstrating the potential of kartogenin as a chondrogenic molecule and its mechanism of action.

Kartogenin

Kartogenin (KGN) is a small heterocyclic molecule discovered by Johnson et al., in 2012 [1] as a potential disease-modifying small molecule for osteoarthritis therapy by regulating chondrogenesis. Using an image-based high-throughput screening of 22,000 structurally diverse hetrocycles using human bone marrow-derived mesenchymal stem cells (hMSCs),

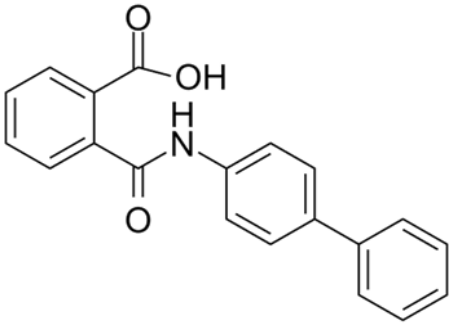

Fig 1: Chemical structure of Kartogenin

KGN was identified for its ability to promote chondrogenesis. The cartilage nodules formed in the presence of KGN contained proteoglycans and collagen II, the essential structural components of the hyaline cartilage. In addition, KGN was able to significantly reduce nitric oxide (NO) and cytokine-induced degradation of glycosaminoglycans (GAGs) in cartilage explants under pathophysiological conditions. Using the collagenase VII-induced chronic joint injury model and acute ligament ligation model, the study also demonstrated the ability of intraarticular injection of KGN to support cartilage regeneration as indicated by a decrease in the fibrillations in the superficial and midzone of articular cartilage. Incapacitance measurements in these models demonstrated the ability of KGN to alleviate OA-induced pain. The study attributed the *in vivo* efficacy of KGN to a combination of regenerative/repair and protective effects.

The above-discussed study significantly increased the interest in KGN as a regenerative molecule and led to the investigation of the molecule for a wide range of biological applications. Kwon at al. investigated the ability of KGN to combat the OA-induced joint pathogenisis [12]. Using mono iodoacetate (MIA) induced inflammatory OA rat model, the study investigated the ability of KGN to reduce pain and improve cartilage repair upon intraarticular injection. The study demonstrated that intraarticular injection of KGN significantly reduced joint destruction, decreased chondroclast expression, increased Treg cell expression, and decreased the severity of joint pain. In addition, using IL-1β stimulated human OA chondrocytes, the study showed the ability of KGN to increase the mRNA levels of anti-inflammatory cytokine IL-10. The authors attributed the reduction of pain, catabolic activity and inflammation by KGN to the upregulation of IL-10. The study attributed the protective activity of KGN towards cartilage degeneration to the downregulation of MMPs, inflammation and reduced chondroclast activity. In addition, the study showed decreased osteoclastogenesis upon KGN treatment. Jia et al., using rabbit synovial fluid-derived mesenchymal stem cells (SF-MSCs) studied the combined effect of KGN with transforming growth factor-β3 (TGF-β3), a known chondrogenic molecule [13]. Addition of KGN significantly increased Type II collagen and SRY-box 9 (SOX-9) expression and decreased Type-X collagen. Moreover, the combined approach showed significantly increased hyaline cartilage regeneration in a rabbit cartilage defect model. Using cartilage stem/progenitor cells, another study investigated the transcriptomic changes upon KGN treatment [14]. In addition to enhancing cell proliferation, KGN treatment led to increased expression of IL-6 and its coreceptor Gp130 as well as phosphorylation of Stat3. Intraarticular injection of KGN in rat medial meniscus transection model resulted in increased articular cartilage thickness and showed upregulated Stat3 phosphorylation and increased presence of CD55/CD105 cells in the cartilage in the presence of KGN. The regulation of matrix metalloproteases (MMPs) is extremely important to maintain cartilage homeostasis as MMPs are known to cause matrix degradation. The ability of KGN to modulate matrix degradation enzymes to reduce cartilage degeneration was recently studied [15]. Using interleukin-1β (IL-1β) treated chondrocytes, the study showed the ability of KGN to maintain a balance between anabolism and catabolism by increasing matrix (type-II collagen and aggrecan) production and reducing the expression of matrix-degrading enzymes (MMP13 and ADAMTS5). In an OA mouse model, the study showed the ability of KGN to ameliorate cartilage degeneration and subchondral bone sclerosis *in vivo*. A related study investigated the effect of KGN on chondrogenesis, by exposing adipose-derived stem cells (ADSCs) to KGN-pretreated ADSC derived exosomes [16]. The pretreated exosomes significantly increased the proliferation, clone formation, migration and chondrogenic differentiation of ADSCs. This study also showed the ability of KGN to balance the anabolic and catabolic response by increasing matrix production and decreasing the production of matrix-degrading enzymes. Structurally, KGN consists of 4-aminobiphenyl (4-ABP) and phthalic acid (PA) held together via an amide bond. A recent study showed that the hydrolysis of KGN and subsequent introduction of 4-ABP into cartilage

can promote MSC proliferation and chondrogenic differentiation [17]. A stronger effect of 4-ABP in increasing CD44+/CD105+ stem-cell recruitment and prevention of matrix loss compared to KGN was demonstrated.

Apart from the effect of KGN on chondrogenesis, its biological effect on other tissues were also investigated. For instance, one study investigated the potential of KGN to regulate the limb developmental process [18]. Using mouse embryo limb buds, the study demonstrated the ability of KGN to stimulate cartilage nodule formation and boost digit cartilaginous anlage elongation, synovial joint formation and interzone compaction, tendon maturation and interdigit invagination. Interestingly, in addition to chondrogenesis, a recent study showed the ability of KGN to improve the osteogenesis of bone marrow MSCs [19]. The study showed a significant increase in autophagy activities and autophagy-related genes, as well as upregulation of phosphorylated Smad1/5/9 signalling in bone marrow MSCs upon KGN treatment. Another study investigated the potential of KGN to support chondrogenic differentiation of patellar tendon stem/progenitor cells [20]. Apart from supporting chondrogenic differentiation of tendon progenitors, KGN also induced cartilage-like tissue formation upon injection into intact rat patellar tendons, and injured rat Achilles tendon-bone joints. Using a rat tendon graft-bone tunnel model, KGN was shown to promote the formation of fibrocartilage zones between a tendon graft and the bone interface, when combined with platelet-rich-plasma (PRP) as a carrier [21]. Due to the phenotypic similarity of MSCs and dermal fibroblasts, Wang et al., studied the effect of KGN on dermal fibroblasts [22]. KGN significantly enhanced type-I collagen synthesis without causing fibroblast apoptosis. Lack of expression of á-skeletal muscle actin, matrix metallopeptidase 1 (MMP1) and MMP9 was also demonstrated. In addition to this favourable *in vitro* response, dermal administration of KGN using microneedles demonstrated the ability of KGN to increase dermal thickness and collagen deposition significantly. Since previous studies have shown the association of Filamin A with Smad5, the study proposed that KGN dissociates CBFâ and smad5 from FLNA, thereby activating the Smad4/smad5 of the TGF-β signalling pathway without changes in the MAPK signalling pathway. The study demonstrated the potential of KGN for wound healing and cosmetic applications.

Sustained KGN delivery systems

Due to the established biological activities of KGN, several studies investigated the potential of developing sustained KGN delivery systems to maximise its biological potency. Most of these are designed as local delivery systems to minimise systemic exposure. Injectable formulations are very attractive, particularly for intraarticular delivery to treat OA. One of the earliest studies in this direction investigated the efficacy of intraarticular injection of KGN-conjugated chitosan nano and microparticles to support cartilage regeneration[23]. The particles showed sustained release of KGN for 7 weeks. The improved efficacy of nanoparticles compared to microparticles in supporting chondrogenesis was also demonstrated. Using a surgically induced OA model, the study demonstrated the reduced degenerative changes with KGN conjugated nano or microparticle intraarticular injection. Similar effects were reported by using KGN-polyurethane (KGN-PU) nanoparticles [24]. Intraarticular injection of KGN-PU nanoparticles significantly reduced cartilage degeneration and showed reduced OARSI scores. Su et al. demonstrated the feasibility of developing a pH-responsive nano-level micelle composed of acid-responsive methoxy poly(ethylene oxide)-hydrozone-poly(εcaprolactone) (mPEG-Hz-b-PCL) [25]. The micelle formulation showed more than 90% of KGN release within 5 h at pH 7.4, showing the need to further optimise the carrier for prolonged release. In another study, a cartilage targeting KGN nanocarrier was developed using multi-arm cationic nano-construct of Avidin (mAv) [26]. The uniqueness of the particle allowed rapid penetration of the carrier into the cartilage matrix. The versatility of the system allowed sustained KGN release

for over 2 weeks using a releasable ester linker (mAV-OH-KGN) and a non-releasable formulation using an amide linker (mAV-NH-KGN) wherein the chondrocytes will take up the nanoparticle. The *in vitro* efficacy of the formulation was demonstrated using a cartilage explant model wherein cytokine-induced catabolism was suppressed. Another recent study investigated the potential of iron oxide nanoparticles to guide intracartilaginous delivery of KGN magnetically [27]. Iron oxide nanoparticles showed sustained release of KGN for over 10 days. The bioactivity of the nanoparticles was established *in vitro* as it supported chondrogenic differentiation of MSCs. In addition, the magnetic particles showed improved tissue penetrating properties in the presence of a magnetic field. Using an OA rat model, the study showed the ability of the particles to reduce cartilage degeneration and alleviate inflammation. Hydrogel carriers are another attractive drug delivery system for cartilage regeneration. Shi et al. developed an injectable composite carrier consisting of KGN-loaded PLGA nanoparticles encapsulated in hyaluronic acid hydrogel [28]. The composite carrier showed sustained delivery of KGN for more than 60 days. The ability of the composite carrier to support hyaline cartilage formation, as well as significant BMSCs and SMSCs homing to the site, was demonstrated using a full-thickness cartilage defect in a rabbit model. Chen et al. developed a theranostic system composed of KGN/synthetic melanin nanoparticles and cellulose nanocrystal/gelatin-methacrylate anhydride (GelMA)/Hyaluronic acid-methacrylic anhydride) hydrogel [29]. In addition to good mechanical properties and magnetic resonance imaging contrast enhancement, the composite matrix showed sustained release for several days. Yuan et al. studied the efficacy of ultrasound-responsive hydrogel as a carrier for KGN for cartilage regeneration [30]. Here, KGN-loaded PLGA microparticles were embedded in carboxymethyl chitosan-oxidized chondroitin sulfate. The microparticles showed collapse under ultrasound and showed sustained release of KGN for about 28 days. A recent study incorporated KGN in electrospun polycaprolactone (PCL) and poly(lactic-co-glycolic acid) (1:1) blends [31]. The incorporation of KGN did not affect the spinnability of the blend, nor did it affect the fibre morphology or mechanical properties. The electrospun fibres displayed a typical biphasic release profile with a sustained release lasting up to 28 days. These studies show the feasibility of local sustained delivery of KGN using biomaterial carriers for improved efficacy to support cartilage regeneration.

Mechanism of Action

Several mechanisms of action have been identified to support the chondrogenic effects of KGN.

Filamin A/CBFβ/RUNX1 pathway for chondrogenesis

Structure-activity-relationship study by Johnson et al., identified association of KGN with FilaminA, an actin-binding protein, which is known to play a key role in cytoskeleton formation by cross-linking actin filaments, anchoring several proteins in the cytoskeleton, and controlling cell adhesion and migration [32]. KGN did not show a significant effect on G-actin (monomeric) or F-actin (filamentous), nor did it affect the distribution of FLNA in these two fractions [1], KGN was shown to block the interaction between CBF-β and FC-1 (a fragment of FLNA) causing translocation of CBF-β into the nucleus to interact with RUNX1 and transcribe genes involved in the chondrogenesis of MSCs [1]. The study, therefore, concluded that RUNX1 may be the downstream effector of KGN. CBF-β is known to activate RUNX1, a transcription factor that promotes cartilage formation by enhancing chondrogenic differentiation of MSCs through transcriptional induction of COL2A1 while suppressing hypertrophic differentiation [33]. Additionally, it was reported that RUNX1 promotes chondrogenic differentiation and cartilage matrix production through its interaction with Sox5, Sox6, and Sox9, members of the SOX family [33]. Sox5,

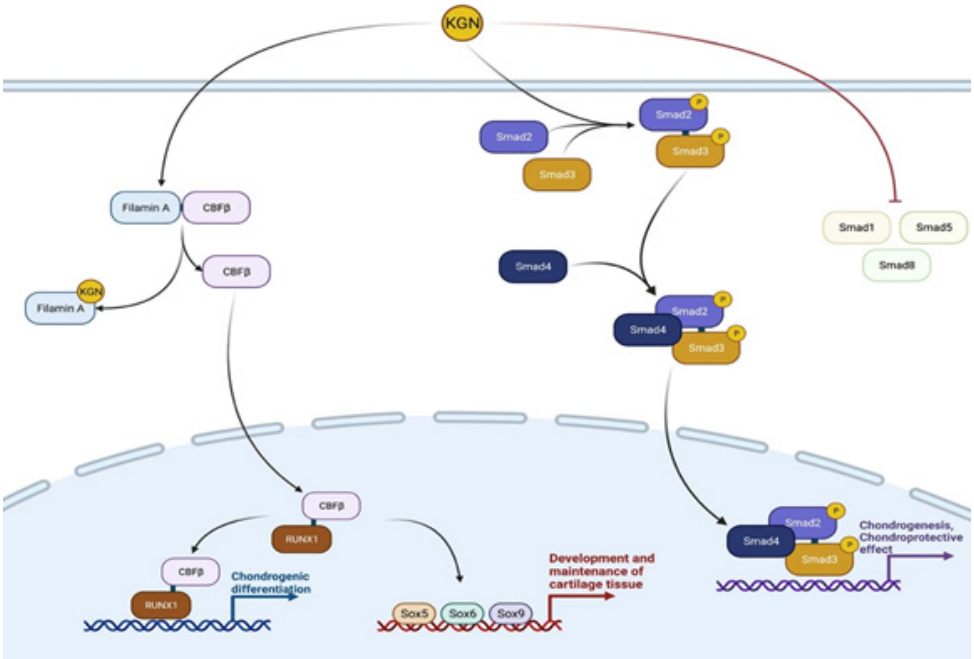

Fig 2: Mechanism of action of Kartogenin *via* the Filamin A/CBFb/RUNX1 Pathway (left) and the TGF-β/Smad Pathway (right)

Sox6, and Sox9 are specifically involved in the development and maintenance of cartilage tissue [34]. They are known to function by interacting with SOX DNA motifs that are part of enhancer regions which are predominantly linked to cartilage-specific genes and thus regulate the expression of various genes involved in chondrogenesis, including genes encoding for extracellular matrix proteins such as collagens and proteoglycans [35]. The functions of Sox5, Sox6, and Sox9 suggest that they play important roles in the development and maintenance of cartilage tissue. An important role of this pathway is the suppression of hypertrophic differentiation by down-regulation of RUNX2, the main activator of hypertrophic chondrocyte differentiation. One study showed that Runx1 possibly suppressed hypertrophic chondrocytes in a Bapx1-dependent manner, where Bapx1 has a partial role in the suppression of hypertrophic differentiation. A previous study showed that Sox9 can directly interact with Bapx1 and promote its gene expression, which in turn down-regulates RUNX2 expression [36] by directing RUNX2 to the lysosome for degradation [37]. Another plausible way that RUNX2 could be suppressed is through an autoregulation method. Prior studies have shown that both canonical and non-canonical RUNX2 genomic sites contribute to the autoregulation of the RUNX2 [38]. As all members of the RUNX family recognize the same DNA sequence, it is plausible that the CBFâ/RUNX1 complex in the presence of KGN suppresses RUNX2 transcription through competitive inhibition, thus keeping RUNX2 at a relatively low level [1,39].

Indian Hedgehog (Ihh) pathway for chondrogenesis

The Indian hedgehog (Ihh) pathway plays a critical role in the development and maintenance of cartilage tissue. Ihh is a key signaling protein secreted by pre-hypertrophic chondrocytes in the growth plate that is needed to sustain chondrocyte proliferation. Ab-

sence of this protein prevents the formation of synovial joints in embryos [18]. The Ihh pathway is activated by the binding of the Ihh protein to the Patched-1 (PTCH1) receptor on the cell surface. This, releases and activates Smoothened (Smo). Smo then translocates to the cilium and recruits the suppressor of fused homologue Sufu-Gli complex to the cilium. The Smo then dissociates the bond between Gli and Sufu, thereby allowing the activated Gli2/3 to enter the nucleus and activate the expression of the Ihh target genes including Ptch1 and Gli [40]. Treatment of isolated forelimb bud pairs from mouse embryos treated with KGN resulted in the upregulation of Ihh target genes. Moreover, the Ihh signaling by KGN was prevented by the co-treatment of KGN with cyclopamine (CPN), a hedgehog chemical inhibitor [18].

TGF-β/Smad Pathway for Chondrogenesis

The TGF-β signalling pathway is another important regulatory pathway for skeletogenesis and joint development. More specifically, this pathway is also responsible for chondrogenesis and lubricin expression [41]. TGF-β activates multiple cell receptors that phosphorylate the Smad secondary messenger proteins, including Smad2/3 and Smad1/5/8. Smad2/3 is responsible for protecting against chondrocyte hypertrophy while Smad1/5/8 cause chondrocyte hypertrophy and OA-like pathogenesis [42]. Kartogenin was shown to impact TGF-β/Smad pathway by promoting phosphorylation and subsequent activation of Smads. KGNs unique activities lie in its ability to activate Smad2/3 resulting in chondroprotective effect [18]. The combination of Kartogenin and TGF-β3 has been shown to more effectively stimulate the chondrogenic differentiation of synovial fluid-derived MSCs than through their individual use [13]. This effect is established via a greater promotion of the Smad2/3 pathway through their combined effect, while KGN inhibits the hypertrophy caused by TGF-β3 via the SMad1/5/8 pathway.

AKT/PI3K and TAOK1/Hippo Pathways

In addition to the pathways discussed above, recent studies showed the role of KGN in other mechanisms mediated via AKT/P13K and the TAOK1/Hippo Pathways. The ability of KGN in combination with PRP to significantly reduce the inflammatory response and cause enhanced wound healing is attributed to the inhibition of AKT/PI3K/NF-κB pathway [43]. The exact details of the mechanism of action are not completely elucidated. The Hippo Signaling pathway is another known pathway involved in regulating differentiation and development [44-46]. Small extracellular vesicles derived from KGN-preconditioned mesenchymal stem cells have shown to enhance chondrogenesis. Using high throughput sequencing, the underlying chondrogenic mechanism of action was studied. The chondrogenesis was attributed to one of the most abundant miRNAs, miR-381-3p, in extracellular vesicles derived from KGN-pretreated cells. Dual-Luciferase reporter study demonstrated the direct suppression of TAOK1 and the Hippo signalling pathway [47]. The study demonstrated yet another KGN pathway in modulating chondrogenesis.

Anti-inflammatory effect

Inflammation in OA has mostly been attributed with interleukin-1 beta ((IL-1β) [48]. IL-1β is an early-stage inflammatory cytokine that induces the activation of multiple pathways, such as NF-κB, PI3K/AKT, and MAPK. These pathways further support the release of other inflammatory molecules. Subsequently, matrix-degrading enzymes such as matrix metalloproteinase-13 (MMP-13) and metalloproteinase with thrombospondin motifs-5 (ADAMTS5) may be secreted and further contribute to the cartilage degradation in OA [49,50] .

In this aspect, treatment with KGN has shown to enhance the synthesis of type II collagen and aggrecan in a dose-dependent manner in chondrocytes treated with IL-1β [15].

Interestingly, this effect is only seen under inflammatory environments, whereas in healthy chondrocytes KGN seemed to have little to no effect on type II collagen and aggrecan levels. Additionally, KGN has a role in suppressing MMP-13 and ADAMTS5 which thereby protects the cartilage extracellular matrix [15] and addresses inflammation.

Another mechanism of inflammation in OA is attributed to the high levels of superoxide anion radical (O2-), hydroxyl radical (HO-), and hydrogen peroxide produced in an OA joint (H_2O_2) [38]. Nuclear factor erythroid 2-related factor 2 (NRF2), a key transcription factor that regulates more than 200 cytoprotective genes [15], has been shown to enter the nucleus in response to oxidative stress. There it binds to an antioxidant response element (ARE) and triggers the transcription of antioxidant enzymes [51]. It has been shown that overexpressing NRF2 prevents the apoptosis and mitochondrial dysfunction brought on by IL-1β in human chondrocytes [52]. As KGN binds to and inhibits miR-146a, an NRF2 inhibitor, it helps reduce the oxidative stress in the cartilage matrix and ameliorates inflammation by allowing for a reduction of Radical Oxygen Species. Overall, the study showed that KGN-mediated cytoprotection is through the reduction of oxidative stress, rescue of structural matrix proteins, and suppression of matrix-degrading enzymes.

Analgesic Effect

Intra-articular injections of KGN in OA mouse models showed a significant reduction of pain as measured by the distribution of weight between the treated and untreated legs [1]. Jiang et al. evaluated the efficacy of intraarticular (IA) KGN treatment in an OA-induced rat pain model using a magnetically guided biodegradable nanocarrier delivery system and found an improvement to the duty cycle, print area, stance phrase and swing speed using a Cat Walk system. These results imply that KGN is effective in treating OA-induced pain [27]. Similarly, another study found a significant decrease in the paw withdrawal latency (PWL), paw withdrawal threshold (PWT) and improvement in the weight distribution of MIA-induced OA rat models upon IA injection of KGN. This study concluded that the KGN injection significantly decreased pain severity in these OA models [1]. The significant decrease in pain molecules such as CGRP and MCP-1 in dorsal root ganglion in KGN-treated rats further supports this. The study attributed the observed significant pain relief to the increase in the expression of IL-10 and Treg cell differentiation upon KGN treatment. Overall, intraarticular KGN seems to be an effective treatment for pain management.

Conclusions

In vitro studies and *in vivo* preclinical studies clearly demonstrated the positive effects of KGN in supporting chondrogenesis, balancing anabolic and catabolic response, reducing inflammation and providing pain relief, thereby highlighting the potential advantages of intra-articular KGN delivery for treating OA. In addition to using KGN alone, a combination of KGN with other chondrogenic molecules also raises significant interest due to their potential synergistic effects. The promising preclinical studies warrant further fundamental studies to thoroughly understand the mechanism of action of intra-articular KGN injections. Furthermore, there exists a significant need to optimise novel biomaterial carriers to effectively deliver the molecule intraarticularly for optimized therapeutic efficacy.

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References

1. Johnson, K.; Zhu, S.; Tremblay, M. S.; Payette, J. N.; Wang, J.; Bouchez, L. C.; Meeusen, S.; Althage, A.; Cho, C. Y.; Wu, X.; Schultz, P. G. A Stem Cell–Based Approach to Cartilage Repair. Science 2012, 336 (6082), 717–721. https://doi.org/10.1126/science.1215157.
2. Park, J.; Lee, S. Y. A Review of Osteoarthritis Signaling Intervention Using Small-Molecule Inhibitors. Medicine (Baltimore) 2022, 101 (32), e29501. https://doi.org/10.1097/MD.0000000000029501.
3. Osteoarthritis (OA) | Arthritis | CDC. https://www.cdc.gov/arthritis/basics/osteoarthritis.htm (accessed 2023-05-23).
4. Chen, D.; Shen, J.; Zhao, W.; Wang, T.; Han, L.; Hamilton, J. L.; Im, H.-J. Osteoarthritis: Toward a Comprehensive Understanding of Pathological Mechanism. Bone Res 2017, 5, 16044. https://doi.org/10.1038/boneres.2016.44.
5. Yasuda, T. Cartilage Destruction by Matrix Degradation Products. Mod Rheumatol 2006, 16 (4), 197–205. https://doi.org/10.1007/s10165-006-0490-6.
6. Martel-Pelletier, J.; Barr, A. J.; Cicuttini, F. M.; Conaghan, P. G.; Cooper, C.; Goldring, M. B.; Goldring, S. R.; Jones, G.; Teichtahl, A. J.; Pelletier, J.-P. Osteoarthritis. Nat Rev Dis Primers 2016, 2, 16072. https://doi.org/10.1038/nrdp.2016.72.
7. Kon, E.; Filardo, G.; Drobnic, M.; Madry, H.; Jelic, M.; van Dijk, N.; Della Villa, S. Non-Surgical Management of Early Knee Osteoarthritis. Knee Surg Sports Traumatol Arthrosc 2012, 20 (3), 436–449. https://doi.org/10.1007/s00167-011-1713-8.
8. Abdeen, A. CHAPTER 42: Osteoarthritis of the Major Joints. 17.
9. Ghouri, A.; Conaghan, P. G. Update on Novel Pharmacological Therapies for Osteoarthritis. Ther Adv Musculoskelet Dis 2019, 11, 1759720X19864492. https://doi.org/10.1177/1759720X19864492.
10. Ayhan, E.; Kesmezacar, H.; Akgun, I. Intraarticular Injections (Corticosteroid, Hyaluronic Acid, Platelet Rich Plasma) for the Knee Osteoarthritis. World J Orthop 2014, 5 (3), 351–361. https://doi.org/10.5312/wjo.v5.i3.351.
11. Sabha, M.; Siaton, B. C.; Hochberg, M. C. Lorecivivint, an Intra-Articular Potential Disease-Modifying Osteoarthritis Drug. Expert Opin Investig Drugs 2020, 29 (12), 1339–1346. https://doi.org/10.1080/13543784.2020.1842357.
12. Kwon, J.; Lee, S.-H.; Na, H.-S.; Jung, K.; Choi, J.; Cho, K.; Lee, C.-Y.; Kim, S.; Park, S.-H.; Shin, D.-Y.; Cho, M.-L. Kartogenin Inhibits Pain Behavior, Chondrocyte Inflammation, and Attenuates Osteoarthritis Progression in Mice through Induction of IL-10. Scientific Reports 2018, 8. https://doi.org/10.1038/s41598-018-32206-7.
13. Jia, Z.; Wang, S.; Liang, Y.; Liu, Q. Combination of Kartogenin and Transforming Growth Factor-Â3 Supports Synovial Fluid-Derived Mesenchymal Stem Cell-Based Cartilage Regeneration. Am J Transl Res 2019, 11 (4), 2056–2069.
14. Liu, T.; Li, X.; Wang, T.; Chen, X.; Zhang, S.; Liao, J.; Wang, W.; Zou, X.; Zhou, G. Kartogenin Mediates Cartilage Regeneration by Stimulating the IL-6/Stat3-Dependent Proliferation of Cartilage Stem/Progenitor Cells. Biochem Biophys Res Commun 2020, 532 (3), 385–392. https://doi.org/10.1016/j.bbrc.2020.08.059.
15. Hou, M.; Zhang, Y.; Zhou, X.; Liu, T.; Yang, H.; Chen, X.; He, F.; Zhu, X. Kartogenin Prevents Cartilage Degradation and Alleviates Osteoarthritis Progression in Mice via the MiR-146a/NRF2 Axis. Cell Death Dis 2021, 12 (5., 1–16. https://doi.org/10.1038/s41419-021-03765-x.
16. Xie, A.; Xue, J.; Wang, Y.; Yang, C.; Xu, M.; Jiang, Y. Kartogenin Induced Adipose-Derived Stem Cell Exosomes Enhance the Chondrogenic Differentiation Ability of Adipose-Derived Stem Cells. Dis Markers 2022, 2022, 6943630. https://doi.org/10.1155/2022/6943630.
17. Zhang, S.; Hu, P.; Liu, T.; Li, Z.; Huang, Y.; Liao, J.; Hamid, M. R.; Wen, L.; Wang, T.; Mo, C.; Alini, M.; Grad, S.; Wang, T.; Chen, D.; Zhou, G. Kartogenin Hydrolysis Product 4-Aminobiphenyl Distributes to Cartilage and Mediates Cartilage Regeneration. Theranostics 2019, 9 (24), 7108–7121. https://doi.org/10.7150/thno.38182.
18. Decker, R. S.; Koyama, E.; Enomoto-Iwamoto, M.; Maye, P.; Rowe, D.; Zhu, S.; Schultz, P. G.; Pacifici, M. Mouse Limb Skeletal Growth and Synovial Joint Development Are Coordinately Enhanced by Kartogenin. Dev Biol 2014, 395 (2), 255–267. https://doi.org/10.1016/j.ydbio.2014.09.011.
19. Yan, H.; Yu, T.; Li, J.; Zhang, T.; Li, Q.; Zhou, Y.; Liu, D. Kartogenin Improves Osteogenesis of Bone Marrow Mesenchymal Stem Cells via Autophagy. Stem Cells Int 2022, 2022, 1278921. https://doi.org/10.1155/2022/1278921.
20. Zhang, J.; Wang, J. H.-C. Kartogenin Induces Cartilage-like Tissue Formation in Tendon-Bone Junction. Bone Res 2014, 2, 14008-. https://doi.org/10.1038/boneres.2014.8.
21. Zhou, Y.; Zhang, J.; Yang, J.; Narava, M.; Zhao, G.; Yuan, T.; Wu, H.; Zheng, N.; Hogan, M. V.; Wang, J. H.-C. Kartogenin with PRP Promotes the Formation of Fibrocartilage Zone in the Tendon–Bone Interface. Journal of Tissue Engineering and Regenerative Medicine 2017, 11 (12), 3445–3456. https://doi.org/10.1002/term.2258.
22. Wang, J.; Zhou, J.; Zhang, N.; Zhang, X.; Li, Q. A Heterocyclic Molecule Kartogenin Induces Collagen Synthesis of Human Dermal Fibroblasts by Activating the Smad4/Smad5 Pathway. Biochemical and Biophysical Research Communications 2014, 450 (1), 568–574. https://doi.org/10.1016/j.bbrc.2014.06.016.
23. Kang, M. L.; Ko, J.-Y.; Kim, J. E.; Im, G.-I. Intra-Articular Delivery of Kartogenin-Conjugated Chitosan Nano/Microparticles for Cartilage Regeneration. Biomaterials 2014, 35 (37), 9984–9994. https://doi.org/10.1016/j.biomaterials.2014.08.042.
24. Fan, W.; Li, J.; Yuan, L.; Chen, J.; Wang, Z.; Wang, Y.; Guo, C.; Mo, X.; Yan, Z. Intra-Articular Injection of Kartogenin-Conjugated Polyurethane Nanoparticles Attenuates the Progression of Osteoarthritis. Drug Deliv 2018, 25 (1), 1004–1012. https://doi.org/10.1080/10717544.2018.1461279.
25. Su, W.-T.; Huang, C.-C.; Liu, H.-W. Evaluation and Preparation of a Designed Kartogenin Drug Delivery System (DDS) of Hydrazone-Linkage-Based PH Responsive MPEG-Hz-b-PCL Nanomicelles for Treatment of Osteoarthritis. Front Bioeng Biotechnol 2022, 10, 816664. https://doi.org/10.3389/fbioe.2022.816664.
26. He, T.; Shaw, I.; Vedadghavami, A.; Bajpayee, A. G. Single-Dose Intra-Cartilage Delivery of Kartogenin Using a Cationic Multi-Arm Avidin Nanocarrier Suppresses Cytokine-Induced Osteoarthritis-Related Catabolism. Cartilage 2022, 13 (2), 19476035221093070. https://doi.org/10.1177/19476035221093072.
27. Jiang, Z.; Zhang, Z.; Li, S.; Lin, S.; Yuan, H. Magnetically Guided Intracartilaginous Delivery of Kartogenin Improves Stem Cell-Targeted Degenerative Arthritis Therapy. Int J Nanomedicine 2022, 17, 5511–5524. https://doi.org/10.2147/IJN.S381815.
28. Shi, D.; Xu, X.; Ye, Y.; Song, K.; Cheng, Y.; Di, J.; Hu, Q.; Li, J.; Ju, H.; Jiang, Q.; Gu, Z. Photo-Cross-Linked Scaffold with Kartogenin-Encapsulated Nanoparticles for Cartilage Regeneration. ACS Nano 2016, 10 (1), 1292–1299. https://doi.org/10.1021/acsnano.5b06663.
29. Chen, C.; Huang, S.; Chen, Z.; Liu, Q.; Cai, Y.; Mei, Y.; Xu, Y.; Guo, R.; Yan, C. Kartogenin (KGN)/Synthetic Melanin Nanoparticles (SMNP) Loaded Theranostic Hydrogel Scaffold System for Multiparametric Magnetic Resonance Imaging Guided Cartilage Regeneration. Bioeng Transl Med 2022, 8 (1), e10364. https://doi.org/10.1002/btm2.10364.
30. Yuan, F.-Z.; Wang, H.-F.; Guan, J.; Fu, J.-N.; Yang, M.; Zhang, J.-Y.; Chen, Y.-R.; Wang, X.; Yu, J.-K. Fabrication of Injectable Chitosan-Chondroitin Sulfate Hydrogel Embedding Kartogenin-Loaded Microspheres as an Ultrasound-Triggered Drug Delivery System for Cartilage Tissue Engineering. Pharmaceutics 2021, 13 (9), 1487. https://doi.org/10.3390/pharmaceutics13091487.
31. Elder, S.; Roberson, J. G.; Warren, J.; Lawson, R.; Young, D.; Stokes, S.; Ross, M. K. Evaluation of Electrospun PCL-PLGA for Sustained Delivery of Kartogenin. Molecules 2022, 27 (12), 3739. https://doi.org/10.3390/molecules27123739.
32. Zhou, J.; Kang, X.; An, H.; Lv, Y.; Liu, X. The Function and Pathogenic Mechanism of Filamin A. Gene 2021, 784, 145575. https://doi.org/10.1016/j.gene.2021.145575.
33. Yano, F.; Ohba, S.; Murahashi, Y.; Tanaka, S.; Saito, T.; Chung, U. Runx1 Contributes to Articular Cartilage Maintenance by Enhancement of Cartilage Matrix Production and Suppression of Hypertrophic Differentiation. Sci Rep 2019, 9, 7666.

https://doi.org/10.1038/s41598-019-43948-3.
34. Lefebvre, V.; Behringer, R. R.; de Crombrugghe, B. L-Sox5, Sox6 and Sox9 Control Essential Steps of the Chondrocyte Differentiation Pathway. Osteoarthritis Cartilage 2001, 9 Suppl A, S69-75. https://doi.org/10.1053/joca.2001.0447.
35. Liu, C.-F.; Lefebvre, V. The Transcription Factors SOX9 and SOX5/SOX6 Cooperate Genome-Wide through Super-Enhancers to Drive Chondrogenesis. Nucleic Acids Res 2015, 43 (17), 8183–8203. https://doi.org/10.1093/nar/gkv688.
36. Yamashita, S.; Andoh, M.; Ueno-Kudoh, H.; Sato, T.; Miyaki, S.; Asahara, H. Sox9 Directly Promotes Bapx1 Gene Expression to Repress Runx2 in Chondrocytes. Exp Cell Res 2009, 315 (13), 2231–2240. https://doi.org/10.1016/j.yexcr.2009.03.008.
37. Cheng, A.; Genever, P. G. SOX9 Determines RUNX2 Transactivity by Directing Intracellular Degradation. J Bone Miner Res 2010, 25 (12), 2680–2689. https://doi.org/10.1002/jbmr.174.
38. Bolduc, J. A.; Collins, J. A.; Loeser, R. F. Reactive Oxygen Species, Aging and Articular Cartilage Homeostasis. Free Radic Biol Med 2019, 132, 73–82. https://doi.org/10.1016/j.freeradbiomed.2018.08.038.
39. Yoshida, N.; Ogata, T.; Tanabe, K.; Li, S.; Nakazato, M.; Kohu, K.; Takafuta, T.; Shapiro, S.; Ohta, Y.; Satake, M.; Watanabe, T. Filamin A-Bound PEBP2â/CBFâ Is Retained in the Cytoplasm and Prevented from Functioning as a Partner of the Runx1 Transcription Factor. Mol Cell Biol 2005, 25 (3), 1003–1012. https://doi.org/10.1128/MCB.25.3.1003-1012.2005.
40. Sigafoos, A. N.; Paradise, B. D.; Fernandez-Zapico, M. E. Hedgehog/GLI Signaling Pathway: Transduction, Regulation, and Implications for Disease. Cancers (Basel) 2021, 13 (14), 3410. https://doi.org/10.3390/cancers13143410.
41. Chimal-Monroy, J.; Díaz de León, L. Differential Effects of Transforming Growth Factors Beta 1, Beta 2, Beta 3 and Beta 5 on Chondrogenesis in Mouse Limb Bud Mesenchymal Cells. Int J Dev Biol 1997, 41 1., 91–102.
42. Cai, J.; Zhang, L.; Chen, J.; Chen, S. Kartogenin and Its Application in Regenerative Medicine. CURR MED SCI 2019, 39 1), 16–20. https://doi.org/10.1007/s11596-019-1994-6.
43. Zhang, L.; Zhang, Q.; Cui, L.; Wu, L.; Gao, S. Kartogenin Combined Platelet-Rich Plasma (PRP) Promoted Tendon-Bone Healing for Anterior Cruciate Ligament (ACL) Reconstruction by Suppressing Inflammatory Response Via Targeting AKT/PI3K/NF-ÊB. Appl Biochem Biotechnol 2023, 195 (2), 1284–1296. https://doi.org/10.1007/s12010-022-04178-y.
44. Pan, J.-X.; Xiong, L.; Zhao, K.; Zeng, P.; Wang, B.; Tang, F.-L.; Sun, D.; Guo, H.; Yang, X.; Cui, S.; Xia, W.-F.; Mei, L.; Xiong, W.-C. YAP Promotes Osteogenesis and Suppresses Adipogenic Differentiation by Regulating â-Catenin Signaling. Bone Res 2018, 6 (1), 1–12. https://doi.org/10.1038/s41413-018-0018-7.
45. Xiao, Y.; Hill, M. C.; Zhang, M.; Martin, T. J.; Morikawa, Y.; Wang, S.; Moise, A. R.; Wythe, J. D.; Martin, J. F. Hippo Signaling Plays an Essential Role in Cell State Transitions during Cardiac Fibroblast Development. Dev Cell 2018, 45 (2), 153-169.e6. https://doi.org/10.1016/j.devcel.2018.03.019.
46. Zheng, M.; Jacob, J.; Hung, S.-H.; Wang, J. The Hippo Pathway in Cardiac Regeneration and Homeostasis: New Perspectives for Cell-Free Therapy in the Injured Heart. Biomolecules 2020, 10 (7), 1024. https://doi.org/10.3390/biom10071024.
47. Jing, H.; Zhang, X.; Luo, K.; Luo, Q.; Yin, M.; Wang, W.; Zhu, Z.; Zheng, J.; He, X. MiR-381-Abundant Small Extracellular Vesicles Derived from Kartogenin-Preconditioned Mesenchymal Stem Cells Promote Chondrogenesis of MSCs by Targeting TAOK1. Biomaterials 2020, 231, 119682. https://doi.org/10.1016/j.biomaterials.2019.119682.
48. Vincent, T. L. IL-1 in Osteoarthritis: Time for a Critical Review of the Literature. F1000Res 2019, 8, F1000 Faculty Rev-934. https://doi.org/10.12688/f1000research.18831.1.
49. Wang, M.; Sampson, E. R.; Jin, H.; Li, J.; Ke, Q. H.; Im, H.-J.; Chen, D. MMP13 Is a Critical Target Gene during the Progression of Osteoarthritis. Arthritis Res Ther 2013, 15 (1), R5. https://doi.org/10.1186/ar4133.
50. Sekino, Y.; Takemoto, K.; Murata, D.; Babasaki, T.; Kobatake, K.; Kitano, H.; Ikeda, K.; Goto, K.; Inoue, S.; Hayashi, T.; Taniyama, D.; Shigeta, M.; Kuraoka, K.; Mita, K.; Kaneko, M.; Sentani, K.; Oue, N.; Teishima, J. CD44 Is Involved in Sunitinib Resistance and Poor Progression-Free Survival After Sunitinib Treatment of Renal Cell Carcinoma. Anticancer Res 2021, 41 (10), 4875–4883. https://doi.org/10.21873/anticanres.15301.
51. Done, A. J.; Traustadóttir, T. Nrf2 Mediates Redox Adaptations to Exercise. Redox Biol 2016, 10, 191–199. https://doi.org/10.1016/j.redox.2016.10.003.
52. Yan, Z.; Qi, W.; Zhan, J.; Lin, Z.; Lin, J.; Xue, X.; Pan, X.; Zhou, Y. Activating Nrf2 Signalling Alleviates Osteoarthritis Development by Inhibiting Inflammasome Activation. J Cell Mol Med 2020, 24 (22), 13046–13057. https://doi.org/10.1111/jcmm.15905.