The efficacy and safety of intra-articular injection of hyaluronic acid in the knee and physical therapy agents to treat Kashin-Beck disease: A prospective interventional study

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Abstract. The aim of the present study was to determine whether hyaluronic acid (HA) or physical therapy agents (PTA) can improve functional parameters in patients with knee Kashin-Beck disease (KBD). For 2 years, patients (n=55) were treated with HA weekly for 5 weeks, then received 6th and 7th injections on the 3rd and 6th month, respectively, for 7 injections in total. Patients (n=53) were treated with PTA five times a week for 3 weeks every month for 6 months. The patients were evaluated with the Western Ontario and McMaster University Osteoarthritis Index (WOMAC) and the visual analog pain scale (VAS). Trial registration, ChiCTR-TRC-12002189 (http://www.chictr.org/). During the study, following treatment interruption, pain increased in the PTA group (from a mean value of 85.7±83.8 mm at month 12 to 145.2±128.8 mm at month 18 and 201.3±150.5 mm at month 24), while it remained stable in the HA group (from a mean value of 80.7±70.6 mm at month 12 to 90.1±95.2 mm at month 18 and 82.6±85.3 mm at month 24), with a statistically significant difference in favor of HA at month 18 (P<0.05) and month 24 (P<0.05). Joint stiffness, physical function and total WOMAC showed the same trend as pain. The global efficacy judgments by the patients and the investigators showed a statistically significant difference in favor of HA at month 18 (P<0.05) and month 24 (P<0.05). In conclusion, although all the patients improved in terms of pain and function, HA was superior to PTA alone for pain relief and lasting effect.

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Introduction

Kashin-Beck disease (KBD) is an endemic osteochondropathy, mainly located in Eastern Siberia in Russia, the diagonal broad belt extending from the Northeast to the Southwest of China, affecting over 0.642 million patients with 37,917 million people at risk (1,2). Clinically, the disease manifests as deformed, enlarged interphalangeal joints with shortened fingers, and limited range of motion (ROM) in the joints of the extremities, which develops in 4 stages (3). Similar to other degenerative joint diseases, the large weight-bearing joints are the most degenerated in adult KBD patients, especially the knee joint. The primary aim of the therapeutic management of KBD, referring to osteoarthritis (OA), is pain relief, protection of ROM, and prevention of secondary functional disability and joint damage. No medical intervention has been shown to arrest disease progression or reverse joint damage and few studies can be found on the validity of medical therapy methods at present (4-6). It is therefore important to identify a useful therapeutic regimen for KBD.

Intra-articular injections, such as those with sodium hyaluronic acid (HA), are widely used in the treatment of knee OA. Although HA has been shown to affect chondrocytes, synoviocytes, and the inflammatory process, it has been demonstrated that the primary objective of HA injections is visco-supplementation of the joint, which aims to increase the elastic viscous properties and restoration of the rheological properties of the synovial fluid in the arthritic joints (7). For the treatment with HA, most studies reported that clinical improvement begins with a delayed onset between 2 and 5 weeks, lasting 6 months or up to 1 year (7-9). Literature reviews revealed that few studies observed the effects of HA on KBD, even though the benefits of HA therapy on OA patients are well known (10-12).

Physical therapy agents (PTA) play an important role in the treatment of OA of the knee. Deep heat such as short waves and electrotherapeutic modalities such as interferential therapy are used to treat acute and chronic pain associated with OA (13-19).

Our use of HA started based on empirical evidence, in an attempt to improve mobility and reduce pain in KBD patients, with knee deformity. After witnessing an initial result within the first year, we performed a formal clinical trial, which was undertaken between August 2006 and March 2009. The aim was to study the effects of HA therapy on the clinical signs and evolution of the disease.

Patients and methods

Patients. The present study was performed with the approval of the Xi'an Jiaotong University Ethics Committee and in compliance with the Helsinki Declaration (register no. ChiCTR-TRC-12002189 http://www.chictr.org/).

The study was a clinical trial: two intervention treatments including intra-articular HA injections in the knee and PTA were conducted within 48 months of follow-up. We examined patients with KBD of the knee joint according to the national diagnosis criteria of KBD in China (3,20). All 123 patients had grade II knee KBD, radiologically confirmed according to the analysis of X-ray films of the right hand, knee and hip joints. The patients suffered pain in the affected knee that continued for at least 6 months. Patients were excluded from the study if they had received intra-articular injections in the joint and/or attended physiotherapy sessions for the affected knee, within the 6 months prior to the study, if they had a history of allergy or hypersensitivity to drugs or eggs, or if they were ascertained or suspected to be pregnant or were lactating. Patients were also excluded if they had a known or suspected joint infection or a specific condition (neoplasm, diabetes mellitus, paresis, osteonecrosis, or recent trauma) or poor general health status that would interfere with the functional assessments during the study. Baseline characteristics (weight, height, age, gender and related radiological assessment of the knee and radiological degree) were recorded prior to the treatment. Laboratory assessment (according to standard methods) made at the entry and after 6 months included the following evaluation: routine hematological variables, and functional tests of the kidney and liver.

Treatments. The patients were divided into the HA or PTA group (n=62 and 61 per group, respectively). Each participant was informed with regard to the study and provided signed consent for treatment. In patients with bilateral disease, the more painful knee was treated. Patients in the first group received HA. The test drug was 20 mg sodium hyaluronate (20 mg/2 ml, 500-700,000; Shanghai Qisheng Biological Preparation Co., Ltd, Shanghai, China), (→3)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyluronic acid-(1→) n, $C_{14}H_{20}NNaO_{11}$. Injections were performed by two physicians in an anterolateral approach (along the patellar tendon) with the knee in 90˚ flexion, and an evaluator unaware of each patient's treatment group, not present at the time or place of each weekly injection, assessed the patients for their symptoms and side effects at baseline and at first injection, 1 week later. A volume of 2 ml was injected intra-articularly into each knee joint once a week for five consecutive weeks without local anesthetics. Strict sterile procedures were applied to prevent septic infection. The 6th injection was carried out in the 3rd month and the 7th injection was carried out in the 6th month.

PTA were applied to each patient of the other group, five times a week for 3 weeks every month for 6 months with a series of infrared (IRH-3100; Korea), short-wave diathermy pulsed patterns and interferential therapy. Each of them was continued for approximately 20 sec, for 1 h.

Clinical assessments were made at the start of the study, and at 1, 3, 6, 12, 18 and 24 months.

The patients could use paracetamol (to a maximum of 2 g daily) during the study period as considered appropriate by the physician. The use of NSAIDs was not permitted during the study period; any pretreatment with NSAIDs had to be discontinued 15 days before the start of the study. Patients were withdrawn from the study if a severe reaction to the injections occurred or if there was evidence of an active infection in the injected joint at any time during the study period.

Efficacy parameters. The primary efficacy criterion was joint pain measured using WOMAC A visual analog pain scale (VAS). Secondary efficacy variables included joint stiffness (WOMAC B) and physical function (WOMAC C), total WOMAC, Short Form 36 (SF-36) health survey questionnaire, daily paracetamol consumption, global efficacy judgment by the patient and the investigator using a four-point scale ('How well do you feel the treatment has worked thus far?', i.e., not effective, slightly effective, moderately effective, very effective), presence of effusion or swelling of soft tissue, and tenderness of the signal joint (VAS) assessed by palpation along the joint line.

Safety parameters. Vital signs were recorded at baseline and at every visit. Blood and urine samples were collected at screening, at week 1 and at the end of treatment (week 4) for laboratory safety analyses. Adverse events (AEs) were recorded at each visit and assessed by the investigator. Patients were asked to assess the tolerability of the study treatment globally ('How well did you tolerate the treatment?') at each visit after baseline using a 5-point rating scale (nil, poor, moderate, good, very good). The investigators also provided a judgment on tolerability ('How well do you think the patient tolerated the treatment?') using the same scale. In both groups the following clinical parameters were used to assess the response to the treatment: the ROM of both knees (measurement with a goniometer of active extension and flexion. However, only flexion was measured because none of the patients had restriction of extension), time to walk a distance of 15 m (measured with a stop-watch and reported in sec), amount of soft tissue swelling and synovial effusion (measured by a meter on volar patellar area and noted if patellar click sign was present by bimanual examination).

Statistical analysis. SPSS for Windows software was used for data management and statistical analysis. Primary analyses were conducted by intent-to-treat using the last observation carried forward technique for missing data, with participants analyzed according to their initial assignment. To compare the groups with regard to demographic measurements, the independent samples t-test or Chi-square test were used between the group analyses. The differences between groups were verified by independent samples t-test. The repeated measurement variables in each group (HA or PTA) were analyzed using analysis of variance (ANOVA) (general linear model for

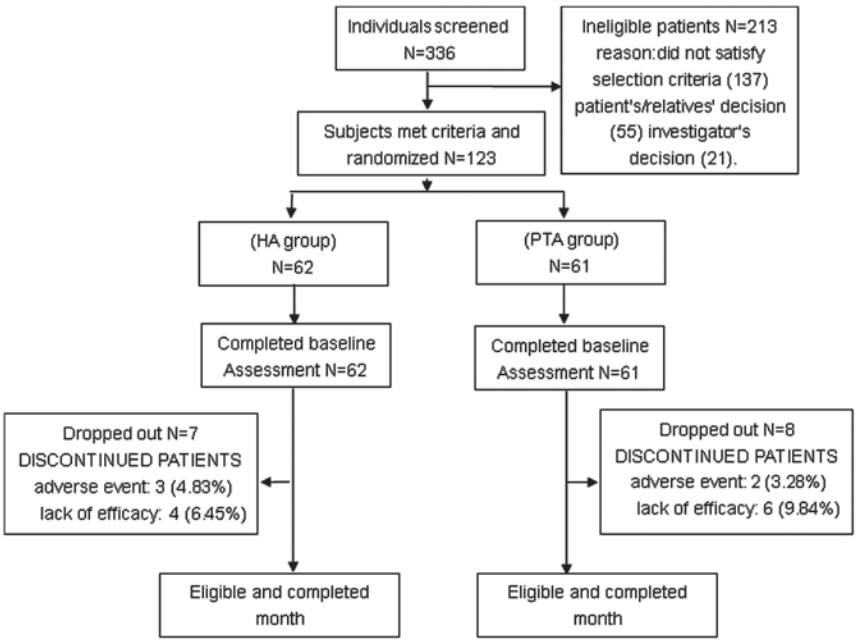

Figure 1. Patient disposition.

repeated measures). The level of significance was set at 0.05 for all statistical tests. All tests of hypotheses and reported P-values were two-sided.

Results

From a pool of 336 individuals, 123 met the screening criteria (Fig. 1). Of the 123 patients, 62 patients were randomized into the HA group and the remaining 61 patients in the PTA group. All participants were given a baseline assessment. Fifteen patients (7 patients in the HA group and 8 patients in the PTA group) dropped out of the study between month 1 and month 24 of assessments due to an adverse event, lack of effectiveness, loss to follow-up or patient decision. A total of 108 patients completed the 24-month study (Fig. 1). The remaining 108 patients (55 in the HA group and 53 in the PTA group) were considered eligible for an effectiveness analysis.

Baseline characteristics. Table I shows baseline demographic data, KBD symptoms, and prior treatment for all the patients eligible for an effectiveness analysis. The two randomized groups were comparable at baseline, and there were no significant differences between the groups for these parameters (P<0.05). In addition, there were no relevant differences in baseline values for the other efficacy parameters (Table I; Fig. 2).

Efficacy results. The mean values and the Student's t-test statistics for the efficacy parameters are shown in Table II. The primary efficacy parameter, pain on WOMAC A, decreased to a similar extent in the two groups during the 12-month treatment period: from a mean value of 285.1±70.0 mm VAS at baseline to 85.7±83.8 mm at 12 months in the PTA group and from 278.0±65.0 mm at baseline to 80.7±70.6 mm at 12 months in the HA group. Although HA appeared to have a faster onset of efficacy at month 1, there were no statistically significant differences between the groups during the treatment period showing that PTA is as effective as HA for pain reduction.

Table I. Demographic and clinical characteristics of patients at baseline.

Characteristics	HA group	PTA group	P-value
N	55	53	
Age (years)	62.4±9.0	58.7±8.3	NS
Female/male (%)	40/15 (72.7/27.3)	35/18 (64.2/34.0)	NS
BMI (kg/m^2)	30.1±5.2	30.9±2.3	NS
Stiffness (min)	9.6±8.2	10.5±6.1	NS
Right/left (%)	36/19 (65.5/34.5)	35/18 (66.0/34.0)	NS
Range of motion (degree)	118.6±12.3	121.3±9.0	NS
Peripheral measurement of knee (m)	42.6±4.1	41.2±2.6	NS

HA group, patients who received intra-articular hyaluronic acid injections; PTA group, patients treated with physical therapy agents. Values are the mean ± SD. NS, not significant; HA, hyaluronic acid; PTA, physical therapy agents.

However, following treatment interruption, pain increased rapidly in the PTA group (from a mean value of 85.7±83.8 mm at month 12 to 145.2±128.8 mm at month 18 and 201.3±150.5 mm at month 24) while it remained stable in the HA group (from a mean value of 80.7±70.6 mm at month 12 to 90.1±95.2 mm at month 18 and 82.6±85.3 mm at month 24) with a statistically significant difference in favor of HA at month 18 (P<0.05) and month 24 (P<0.05) (Table II).

Joint stiffness (WOMAC B), physical function (WOMAC C) and total WOMAC showed the same trend as WOMAC A

Table II. Efficacy parameters-mean absolute values and MW statistics.

WOMAC (mean ± SD)	HA (n=55)	PTA (n=53)
WOMAC A		
Baseline	278.0±65.0	285.1±70.0
Month 1	166.2±84.3[a]	190.5±93.6
Month 3	123.2±85.9[a]	136.1±94.8[a]
Month 6	90.3±74.2[a]	102.0±92.7[a]
Month 12	80.7±70.6[a]	85.7±83.8[a]
Month 18	90.1±95.2[a]	145.2±128.8
Month 24	82.6±85.3[a]	201.3±150.5
WOMAC B		
Baseline	124.8±38.5	125.5±45.6
Month 1	75.2±37.9[a]	89.7±48.5[a]
Month 3	55.3±38.8[a]	65.2±45.0[a]
Month 6	39.6±33.3[a]	45.7±42.1[a]
Month 12	32.6±34.0[a]	38.9±36.7[a]
Month 18	36.3±36.5[a]	64.6±48.9[a]
Month 24	36.1±37.2[a]	56.9±56.2[a]
WOMAC C		
Baseline	875.1±265.3	901.8±252.5
Month 1	556.6±313.1	643.9±354.1
Month 3	453.2±312.5[a]	518.4±311.3[a]
Month 6	326.0±266.0[a]	357.5±310.2[a]
Month 12	245.4±260.2[a]	305.0±279.1[a]
Month 18	313.7±317.6[a]	448.5±403.5[a]
Month 24	297.8 ±322.9[a]	639.0±516.5
WOMAC total		
Baseline	1253.2±354.3	1293.6±343.8
Month 1	768.0±406.6[a]	928.4±481.7
Month 3	603.7±416.7[a]	712.6±482.1
Month 6	451.9±366.4[a]	512.1±451.2[a]
Month 12	357.7±346.1[a]	415.1±424.0[a]
Month 18	441.2±450.4[a]	658.3±586.4
Month 24	421.8±454.0[a]	896.6±652.3
SF-36 (sum score)		
Baseline	358.0±114.2	355.3±121.6
Month 18	556.6±142.3[a]	507.9±136.7[a]
Month 24	542.9±156.6[a]	514.1±146.7[a]

HA group, patients who received intra-articular hyaluronic acid injections. PTA group, patients treated with physical therapy agents. [a]P<0.05 compared with baseline, calculated on the median percent change. Values are the mean ± SD. HA, hyaluronic acid; PTA, physical therapy agents; WOMAC, Western Ontario and McMaster University Osteoarthritis Index.

(Table II; Fig. 2). Tenderness on palpation decreased in the two groups with a significant difference (P<0.05) (Table II).

The global efficacy judgments by the patients and the investigators are presented in Table III and confirm the slow onset of efficacy of HA with 76.4% in this group compared with 56.6% of the patients in the PTA group judging that their treatment was 'moderately effective' to 'very effective' at month 1. The judgments were comparable in the two treatment groups at the end of the treatment. At month 12, a significantly (P<0.001) greater proportion of HA-treated patients (78.1%) assessed treatment as 'moderately effective' to 'very effective', compared to 41.5% of those treated with PTA while at month 24, these figures were 65.4% in the HA group and 30.2% in the PTA group (P<0.001).

Safety. Three patients of the HA group withdrew from the study due to adverse reactions (malaise, tachycardia and hypotension). The events occurred after the first injection. Two cases of adverse event were observed in the PTA patients. No clinically significant changes occurred in the laboratory parameters or other vital signs in either group.

Discussion

HA is a non-sulfated, non-epimerized, linear glycosaminoglycan (GAG) existing *in vivo* as a polyanion of HA and composed of repeating disaccharide units of D-glucuronic acid and N-acetyl-D-glucosamine (->4GlcAbeta1->3GlcNAcbeta1->) (7,8). It is a constituent of the extracellular matrix (ECM) of the skin, joints, eye, and many other tissues and organs. In the joint, HA is an important component of the extracellular matrix of the cartilage (21). It is present in the superficial layers of the synovial membrane and is found at high concentration in the synovial fluid (22-28). HA plays a key role in preserving the structural and functional integrity of the cartilage matrix and in regulating a variety of cellular activities through specific cellular receptors and molecular interactions in addition to maintaining the viscoelastic properties of synovial fluid. *In vitro* and *in vivo* studies have shown that HA can induce proteoglycan synthesis and aggregation, stimulate synoviocytes to produce more HA, modulate the inflammatory response, reduce chemotaxis and leucocyte migration and exert scavenger activity on free oxygen radicals (5). These activities are mediated by the binding of HA with intercellular adhesion molecule-1 (ICAM-1), CD44 integrin and the receptor for HA-mediated motility (RHAMM), all of which are expressed on the surface of various cell types, including inflammatory cells, synoviocytes and chondrocytes. HA injected in the joint has a half-life of approximately 20 h when joints are normal and approximately 12 h when joints are inflamed (29). The above evidence shows that the clinical effects of HA are due to its pharmacological action on the cellular and tissue components of the joint. Therefore, it can be excluded that the sustained beneficial effects of HA on symptoms and clinical signs of KBD, such as OA, can be accounted only for a temporary restoration of the synovial fluid viscoelasticity.

The effect of HA on KBD may be related to inhibiting the levels of cytokines, specific cellular receptors, and molecular interactions. It was reported that sodium HA administration has a dose-dependent effect *in vitro* to promote the proliferation and inhibition of apoptosis of chondrocytes from patients with KBD (30,31). Phenotypic expression of types I, II, III, and X collagen and MMP-13 in chondrocytes cultured *in vitro* were significantly different between the KBD and control cultures, showing degenerative and hypertrophic changes in chondrocytes of KBD articular cartilage (30). Increases of the levels of tumor necrosis factor (TNF)-α, vascular endothelial

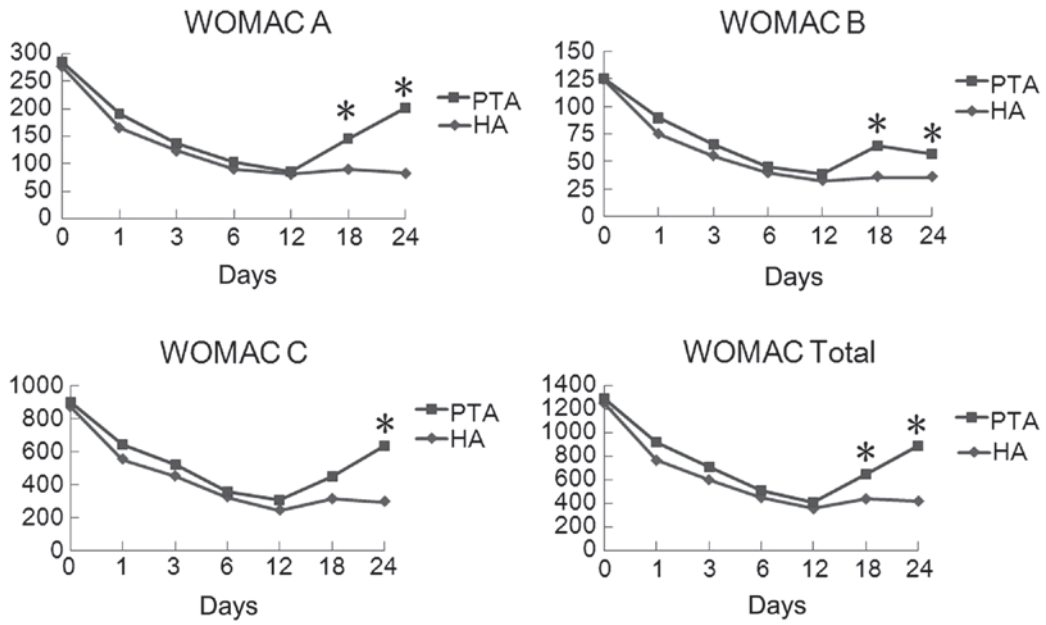

Figure 2. Mean WOMAC subscale levels decreased in all patients followed by an increase for PTA and HA groups. *This decrease was significant between PTA and HA group (P<0.05). HA group, patients who received intra-articular hyaluronic acid injections; PTA group, patients treated with physical therapy agents. Values are the mean ± SD. WOMAC, Western Ontario and McMaster University Osteoarthritis Index; PTA, physical therapy agents; HA, hyaluronic acid.

Table III. Clinical assessments of the drug treatments (global efficacy judgments by the patients and the investigator).

	HA (n=55)		PTA (n=53)		P-value	
Effective (%)	a	b	a	b	a	b
Month 1					0.042	0.144
Not	9.1	11.0	20.8	17.0		
Slightly	14.5	12.7	22.6	24.5		
Moderately	36.4	40.0	28.3	28.3		
Very	40.0	36.4	28.3	30.2		
Month 6					0.003	0.013
Not	7.3	9.1	13.2	11.3		
Slightly	10.9	12.7	35.8	35.8		
Moderately	5.5	40.0	30.2	32.1		
Very	36.4	36.4	20.8	20.8		
Month 12					0.001	0.002
Not	12.7	9.1	28.3	4.5		
Slightly	9.1	12.7	30.2	30.2		
Moderately	43.6	38.2	24.5	22.6		
Very	34.5	8.2	17.0	22.6		
Month 24					0.001	0.001
Not	20.0	21.8	39.6	43.4		
Slightly	14.5	12.7	30.2	26.4		
Moderately	23.6	21.8	13.2	13.2		
Very	41.8	43.6	17.0	17.0		

a, efficacy judgment by the patient. b, efficacy judgment by the investigator. HA group, patients who received intra-articular hyaluronic acid injections. PTA group, patients treated with physical therapy agents. Values are the mean ± SD. HA, hyaluronic acid; PTA, physical therapy agents.

growth factor (VEGF) and interleukin (IL)-1β may play a role in the pathogenesis of KBD. Terminal chondrocytes differentiation, PTHrP, transforming growth factor (TGF)-β1, and VEGF expression was altered showing degenerative changes in KBD cartilage (32-36). Previous findings showed that HA can promote the proliferation of chondrocytes and has

effects on the differentiation of these cells (24-26,37). *In vitro* experiments showed that HA can enhance the synthesis of extracellular matrix proteins, including chondroitin and keratin sulfate, while suppressing cartilage damage by fibronectin fragments *in vitro* and *in vivo* (24,37-40).

The present findings have shown that while an improvement was observed in the functions of all patients, pain intensity decreased more rapidly and to lower levels with the use of HA. In the present study, pain relief by the WOMAC pain subscale was observed in the first month in the two groups. Similarly, this pattern of decrease in pain was also observed by VAS.

Whether HA was used or not, the improvement in function was persistent during the first year. The use of HA injections should be considered for rapid and prolonged effect in the improvement of knee KBD. Our findings support that HA injections could lead to better results in pain reduction.

Our study has several limitations which must be considered. The number of patients studied was small due to medical costs, increasing the power of the study to show significant effects. Second, since there was no other medicine-treated group, such as a saline solution group, perhaps the HA treatment may not be more effective than intra-articular or other drugs. However, the main objective of this study was to assess the effects of HA on the management of knee KBD, not to compare the effects of different drug therapies. Furthermore, HA has been demonstrated to be effective, and has been found to be superior to placebo injections, and comparative studies have shown the differences between timing of onset of effects, as reported above. In conclusion, this study demonstrates that HA provides rapid pain relief, has beneficial effects during the first year following treatment, and is well tolerated in the management of knee KBD. For the treatment of patients with knee KBD, our findings support that HA should be the preferred therapeutic option.

In conclusion, the results of this study support the observation that PTA is a useful, safe and well-tolerated treatment for patients with knee OA, and who are receiving hyaluronan therapy. Although all patients showed improvement for pain and function, HA therapy was superior to PTA alone for pain relief and had a longer lasting effect.

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