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Original contribution

Oral polymeric N-acetyl-D-glucosamine and osteoarthritis

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Many patients with arthritis are using alternative modes of therapy, including nutritional supplements, to treat their arthritis. Most patients never tell their doctors that they are taking alternative medications, and few doctors even ask about such activities. Over-the-counter supplements are expensive and consume large amounts of patients' healthcare dollars. Glucosamine has been widely touted as being an effective arthritis treatment. The authors designed and undertook a study to test the efficacy of a polymer of N-acetyl-D-glucosamine (NAG), or POLY-Nag, in a double-blind, placebo-controlled study in patients with osteoarthritis. Results indicate that POLY-Nag may be useful in treating patients with osteoarthritis.

(Key words: osteoarthritis, glucosamine)

Osteoarthritis (OA) is the most common form of arthritis and a major cause of morbidity and disability in the elderly. Osteoarthritis affects an estimated 12% of the US population, increases with age, and it is found in most persons over the age of 65.[1] Pain is the primary symptom of OA. Traditionally, pain management has relied on acetaminophen and various nonsteroidal anti-inflammatory drugs (NSAIDs). Unfortunately, such therapy does not address the underlying degenerative disorder, and some of these medications can have serious adverse side effects.

A recent survey of arthritis patients[2] found that patients were interested in alternative treatments for arthritis because they wanted pain relief, sought relief of symptoms other than pain, and believed the side effects of alternative treatments would be less than those of prescription medications. In this same survey, glucosamine was found to be the most common supplement used for treating patients with osteoarthritis.

The potential use of glucosamine and its derivatives, such as glucosamine sulfate and N-acetyl-D-glucosamine (NAG), for the treatment of patients with OA has been recognized for several years. One rationale for the use of such products is based on the fact that glucosamine and NAG are the primary building blocks of the structural matrix of connective tissue in joints (for example, glycosaminoglycans [GAGs], chondroitin, and hyaluronic acid). Glucosamine is not only a substrate for synthesis of GAGs, but it also stimulates their synthesis and inhibits their degradation. For example, when glucosamine is added to fibroblasts or chondrocytes in cell culture, it enhances the synthesis of GAGs[3] and inhibits their degradation by suppressing collagenase-1.[4]

In addition to these direct effects on synthesis and degradation of GAGs, another potential mechanism by which glucosamine may exert therapeutic value is by protecting the body from oxidative damage. It is well-established that the levels of reactive oxygen species and oxidatively damaged proteins are elevated in the synovium of patients with arthritis.[5,6] NAG is capable of scavenging reactive oxygen species, such as superoxide anion, hydrogen peroxide, and hydroxyl radical.[7]

In veterinary medicine, glucosamine and chondroitin derivatives have been used successfully for several years to treat symptoms of arthritis. Thus, it was logical that glucosamine and its derivatives would be explored for treating OA in humans. In a review, daCamara and Dowless[8] concluded that although there is evidence for glucosamin's efficacy in relieving pain and increasing mobility in patients with OA, larger and more controlled studies were needed. Several such studies have recently been reported. For example, Leffer and others[9] conducted a 16-week, randomized, double-blind, placebo-controlled crossover trial on degenerative joint disease of the knee and low back.[7] They concluded that a combination of glucosamine HCl (1.5 g/day), chondroitin sulfate (1.2 g/day), and manganese ascorbate (228 mg/day) was efficacious and safe. Qui and coworkers[10] studied 178 patients with OA of the knee in a double-blind protocol comparing treatment for 4 weeks with either daily doses of glucosamine sulfate (1.5 g) or ibuprofen (1.2 g). They concluded that glucosamine was more effective and significantly better tolerated than ibuprofen. Recently, Reginster[11] reported results of a randomized, placebo-controlled, double-blind, 3-year trial with glucosamine sulfate. In this study of 212 patients with OA of the knee, glucosamine prevented narrowing of the joint space, and the glucosamine-

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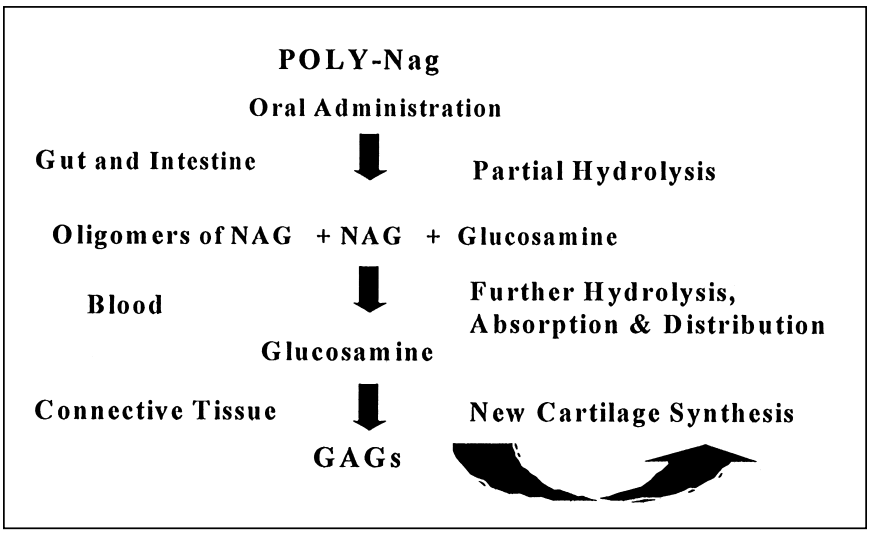

Figure 1. *Potential mechanisms for use of oral POLY-Nag for treatment of patients with osteoarthritis.*[17]

treated subjects showed 25% improvement—while the placebo-receiving control subjects' symptoms worsened.

Although the use of glucosamine and its derivatives for the treatment of patients with OA has a mechanistic basis, and clinical trials continue to substantiate safety and efficacy, remaining issues have included bioavailability, optimal dosage, and delivery. Barclay and others[12] reported that following oral administration of glucosamine, it is incorporated into plasma proteins during first-pass metabolism and results in 26% bioavailability. Unbound glucosamine is incorporated into articular cartilage. Animal and human studies on the absorption, distribution, and excretion of either injected or orally administered radiolabeled GAGs have been conducted.[13-15] Glucosamine exhibited good absolute bioavailability and also was preferentially localized to articular cartilage. However, the half-life of glucosamine in the blood was found to be relatively short. Thus, a sustained-release form of glucosamine would be highly desirable.

We have examined the use of a polymeric form of NAG (chitin, or poly [N-acetylglucosamine] [POLY-Nag, Lescarden Inc, New York, NY]) and found that it may hold promise as a sustained-release, natural form of glucosamine that is both safe and effective in the treatment of patients with OA. Portions of these studies have been published previously[16,17] and form the basis of a patent filed by Gracy and Sherman (Patent No. 08/990,161). *Figure 1* shows the general rationale for the use of this polymer for the sustained release of glucosamine in the treatment of patients with OA.

Materials and methods

POLY-Nag was supplied from Lescarden Inc. Analytical determination of NAG and glucosamine in sera was achieved through high-performance liquid chromatography (HPLC) as follows: Fasting blood samples were obtained by standard venipuncture, and the sera were centrifugally ultrafiltered at 5°C through a 3K membrane. Samples were concentrated tenfold in vacuo in a Speed Vac centrifuge. Ten-microliter aliquots of samples or monosaccharide standards were mixed with 40 μL of benzoic acid ethyl ester (BAEE) derivatization reagent solution, heated at 80°C for 60 minutes, cooled, and extracted with 200 μL of chloroform and 200 μL deionized water. A 50-μL aliquot of the aqueous fraction was separated on a Waters PicoTag column at 45°C using an isocratic gradient of 75% (v/v) 50 mmol sodium acetate buffer, pH 4.5, and 25% (v/v) sodium acetate buffer:acetonitrile:methanol (40/40/20 v/v/v) at a flow rate of 1.2 mL/min.

The bioavailability study was a randomized, blinded, crossover protocol with 10 healthy subjects (5 men, 5 women; aged 36 to 50 years). The clinical trials pilot study involved 10 subjects with OA. The clinical trials pilot study was randomized and double-blinded, and subjects received either 1.5 g of POLY-Nag per day or a placebo for 6 weeks. In the pilot clinical trials, the following dependent variables were measured: OA severity index (SI), doctor/physician global assessment (DG), and patient global assessment (PG).

Monoclonal antibody against unsaturated chondroitin sulfate C (3B3) was prepared according to the method described by Caterson and others.[18] Levels of the 3B3 epitope were analyzed in sera from normal controls (n = 20) and patients in whom osteoarthritis was diagnosed (n = 40) and were compared with levels from subjects participating in the present study. The samples were digested with the chondroitinase ABC, incubated 16 hours at 37°C, followed by heating at 100°C for 10 minutes. All analyses were performed in triplicate with porcine aggrecan core protein (chondroitinase ABC—digested porcine laryngeal cartilage aggrecan) as a standard[18] prior to immunoassay with the monoclonal antibody 3B3.

Results

Bioavailability of glucosamine from POLY-Nag

A fundamental initial question was whether the polymer is degraded and absorbed at sufficient levels to provide glucosamine for synthesis of GAGs. To assess this we used the HPLC analytical system. This allowed the quantitative determination of levels of glucosamine and NAG in the serum. The bioavailability study compared 10 healthy adults. After randomization and a pretest for levels of serum NAG and glucosamine, half of the group ingested NAG (1 g/day) for 3 days while the other half ingested the same amount of POLY-Nag. Following the final ingestion, blood samples were drawn at 1, 2, 4, 8, 24, and 48 hours, and the levels of both NAG and glucosamine were determined. After a

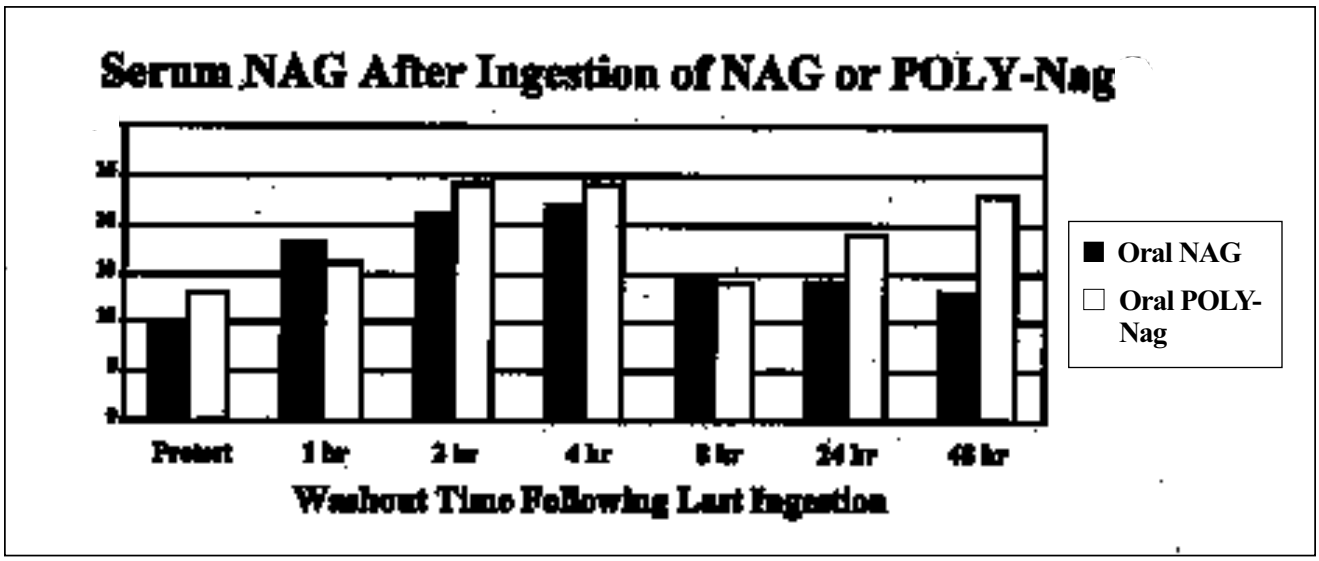

Figure 2. *Effect of oral ingestion of N-acetyl-D-glucosamine (NAG) (closed bars) or POLY-Nag (open bars) on serum NAG concentrations. Serum levels of NAG were determined by HPLC analyses. After 2 days of oral consumption of either NAG or POLY-Nag, a pretest blood sample was taken and a third and final sample was ingested. Levels of serum NAG (nmol/mL* $\times$ *10^{-1}) were determined thereafter and during the following 48-hour washout period.*[16]

further washout period of 2 weeks in which the subjects took neither of the test substances, the crossover was then implemented and the subjects who had received NAG now ingested POLY-Nag, and vice versa.

As seen in *Figure 2*, ingestion of either NAG or POLY-Nag resulted in elevation of NAG. A similar pattern was found with glucosamine. These data indicate that orally administered POLY-Nag is hydrolyzed in vivo to glucosamine, and that both NAG and glucosamine are absorbed.

When subjects stopped ingesting NAG, the levels of serum NAG decreased. In contrast, the serum NAG levels remained elevated for subjects who had been ingesting POLY-Nag. These data suggest that oral ingestion of POLY-Nag was providing a sustained delivery of the aminosugars. No differences were observed on the basis of sex or age of the volunteers. No adverse reactions to ingestion of either NAG or POLY-Nag were evident at the dosage levels used in this study.

These short-term bioavailability studies were encouraging, and thus a second study was undertaken using a different protocol. All of these subjects had existing OA, and the length of time for oral administration was increased. After initial screening and randomization, in a double-blinded study, 10 subjects would receive either POLY-Nag (1.5 g/day) or a placebo.

After randomization, subjects were removed from any medications or dietary supplements containing glucosamine. After 2 weeks, blood samples were obtained and baseline values established. The groups then initiated daily oral ingestion of either POLY-Nag or the placebo. Subjects were reexamined and blood samples collected at 2 weeks and at 6 weeks after the baseline was established. Clinical assessments included patients' self-reported pain, physician's assessment of pain, and an arthritis impact scale of quality of life. Routine laboratory analyses at each visit included complete blood counts, serum chemistries, and urinalysis. In addition, sera were monitored for levels of glucosamine, NAG, and the monoclonal 3B3 epitope for chondroitin sulfate C.

The results of blood analyses showed that four of the five subjects receiving the POLY-Nag had major increases in serum glucosamine and NAG levels. In contrast, four of the five subjects in the placebo group showed decreases in glucosamine levels during the study period. This decrease may represent a continued loss of glucosamine even after the 2-week suspension of intake of glucosamine-containing drugs or food supplements. The overall change in serum glucosamine levels for subjects who received POLY-Nag was positive (+310.4 nmol/mL), while the average for the subjects receiving the placebo was negative (−332.2 nmol/mL). Similarly for NAG levels, the subjects in the POLY-Nag group had an increase of +175.1 nmol/mL while the placebo group decreased (−76.6 nmol/mL).

These data were consistent with the monoclonal antibody studies. Serum levels of the 3B3 epitope for chondroitin sulfate C are indicative of active arthritis. For example, normal controls ($N = 20$) had levels of 14.4 ± 14.1 ng/mL, while patients with OA ($n = 40$) had levels of 80.4 ± 39 ng/mL. In the present study, those subjects who received POLY-Nag had lower levels (46.8 ± 26.8) than the placebo group (84.7 ± 8.9).

Effect of POLY-Nag on clinical symptoms of osteoarthritis

In the same pilot study, blood chemistries performed for each subject measured serum glucose, blood urea nitrogen, cre-

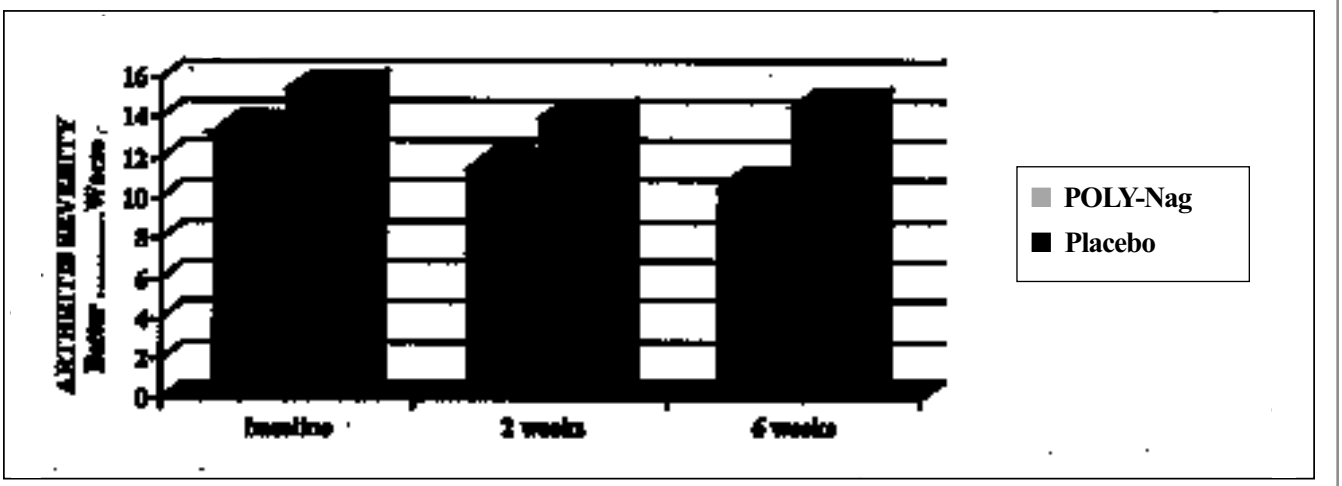

Figure 3. *Effect of POLY-Nag on osteoarthritis severity index.*

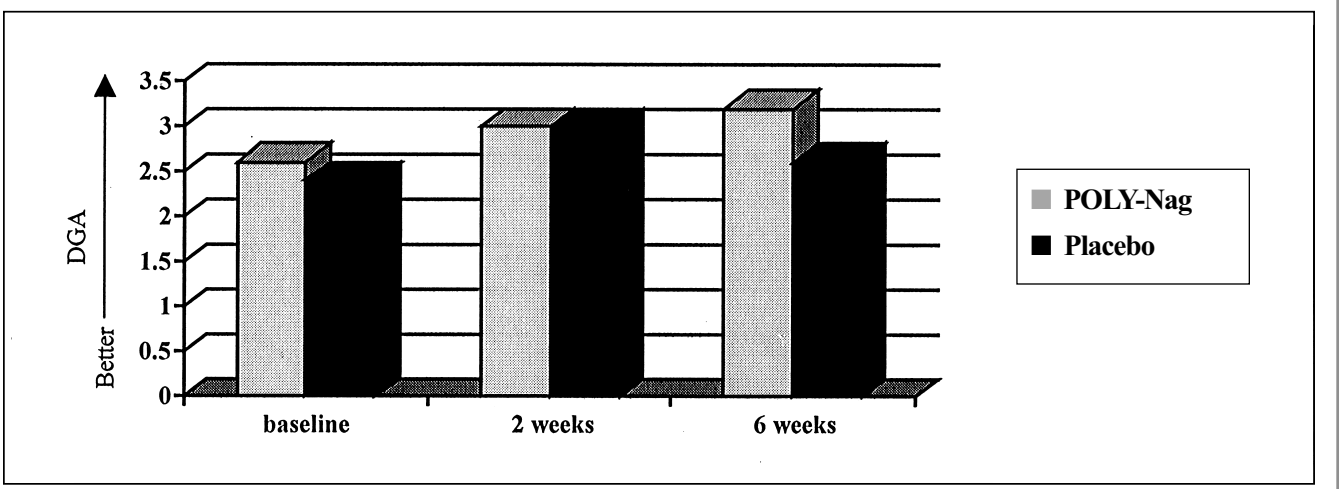

Figure 4. *Effect of POLY-Nag on doctor's global assessment.*

atinine, sodium, potassium, chloride, calcium, ionized calcium, total CO_2, total protein, albumin, globulin, total bilirubin, alkaline phosphatase, and the liver enzymes AST (SGOT). In all cases, the values for these parameters were in normal ranges and did not change significantly during the study. Urinalysis profiles in all subjects were within normal ranges and exhibited no significant changes during the course of the study. Similarly, complete blood count analyses were unremarkable with no significant changes during the course of the study.

However, significant improvement was found in the symptom assessments for the subjects who ingested POLY-Nag. As seen in *Figure 3*, during the 6-week study, the osteoarthritis severity index decreased among the patients receiving POLY-Nag but remained essentially unchanged in the placebo group. Both the doctors' (*Figure 4*) and the patients' (*Figure 5*) global assessments had improved with regard to the POLY-Nag group to a greater degree than for the placebo group. These are visual analogue scales, which reflect overall arthritis symptoms, and the graphs note that a higher value indicates less pain and improved overall function (on a scale from 0 to 10, where we have plotted the differences from baseline).

Comments

Glucosamine has been studied in the treatment of patients with osteoarthritis for more than 15 years. Although there are many anecdotal reports of the benefits of glucosamine, few complete studies have been performed. Many of these studies have not been well controlled, contained too few subjects, and were not rigorous in their assessment of responses. Also, glucosamine is often sold in a combination with another substance called chondroitin sulfate, and there are no clinical data available on the efficacy of this combination.

In considering the possibility of using POLY-Nag for treating patients with OA, it was recognized that chitin, and chitosan-based polymers, have a history of pharmaceutical use in drug delivery. It is

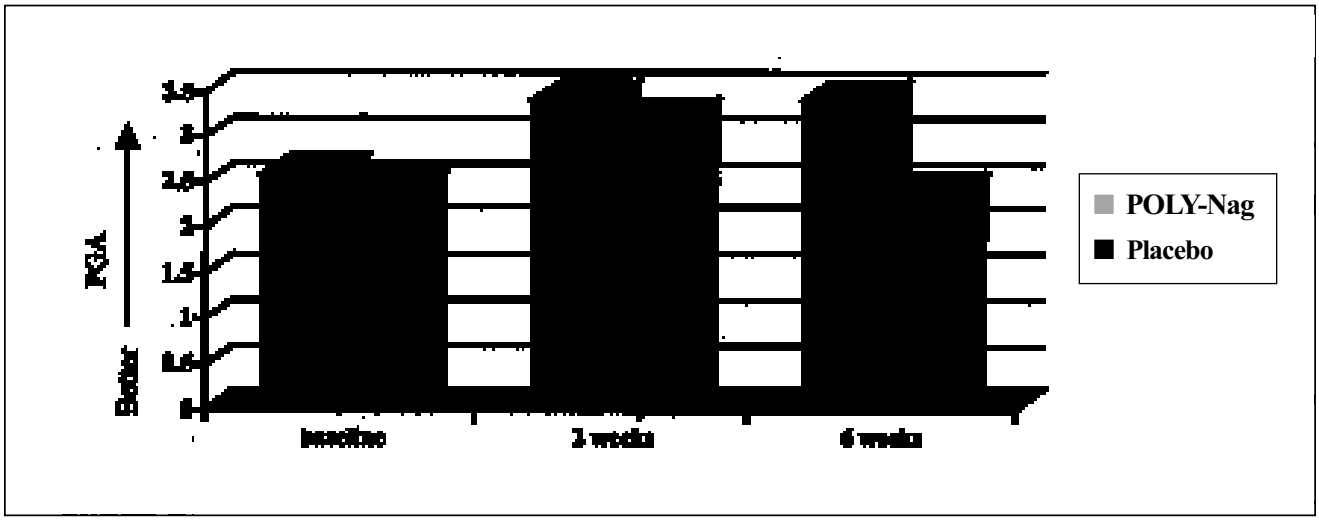

Figure 5. *Effect of POLY-Nag on patient's global assessment.*

important to recognize that chitosan is the deacylated derivative of chitin and that the two polymers have some significantly different properties. For example, the deacetylated chitosan is polycationic, allowing it to bind to macromolecules and cells. Chitin and POLY-Nag are only partially deacylated and have a lower cationic charge/mass ratio.

Genes, peptides, hormones, antibiotics, vaccines, chemotherapeutic drugs, enzyme inhibitors, and many other agents have been delivered orally via chitosan. Its favorable properties include nontoxicity, bioavailability, and biodegradability.[19,20] In addition to its uses described previously, chitosan is reported to have several direct pharmacologic properties. While some of these have not been verified with stringent clinical trials, the polymer has been used as an antacid, as well as an antiuricemic, antiosteoporotic, antimetastatic, and antibacterial agent. Additionally, it has been used in wound healing, as an immunoadjuvant, and in lowering cholesterol.[20]

Although the mechanistic basis for some of chitosan's potential pharmacologic actions are unknown, the efficacy of POLY-Nag in the treatment of patients with OA can be readily explained by several mechanisms. These include its involvement as (1) a substrate for GAG synthesis, (2) an activator of GAG synthesis, (3) an inhibitor of the degradation of connective tissues of the joint, (4) an antioxidant that traps damaging reactive oxygen species, (5) an anti-inflammatory, and (6) a stimulator of synthesis of other components, such as hyaluronic acid.[3]

While it is not known which factors are most important, the data in the present study indicate that POLY-Nag is hydrolyzed to monomers of NAG and glucosamine. A number of enzymes are likely involved in this degradation.[21] Oral ingestion of POLY-Nag is expected to yield oligomers and monomers which are generated by nonspecific lipases, lysozyme, and N-acetyl-glucosaminidase. Also, human chitinase activates macrophages and stimulates fibroblasts.

It appears that POLY-Nag is an effective and safe agent in the treatment of patients with OA. Its bioavailability is documented, and the clinical results of this small pilot study are promising. Further clinical trials need to be conducted to establish optimal dosages. It is also possible that other formulations in which the polymer is partially hydrolyzed before final formulation might be useful.

There are still many unknowns regarding the safety and efficacy of glucosamine. Glucosamine is sold as a dietary supplement without any testing for efficacy, safety, or manufacturing standards. The Dietary Supplement and Health Education Act does not require manufacturers to assess their product to verify that the labeling is accurate. Thus, there is no guarantee that what is on the label is what is actually in the product. There also may be lot-to-lot variations in the quality of the supplements. It is difficult to keep track of the different glucosamine products that are available because they are sold in stores, through direct catalogue sales, over the Internet, and by other means.

As is often the case, patients with osteoarthritis and other chronic diseases are frequently willing to try alternative modes of therapy in the hopes for a cure. It is only through long-term safety and efficacy studies performed by qualified scientists—with the results published in appropriate peer-reviewed journals—that we may learn the real role for glucosamine in the treatment of osteoarthritis.

Acknowledgments

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References

1. Felson DT. Epidemiology of hip and knee osteoarthritis. *Epidemiol Rev* 1988;19:1-28.

2. Horstman J. The dangerous divide: alternative attitudes survey—what arthritis doctors and patients really think. *Arthritis Today* 1999; Nov-Dec:34-41.

3. McCarty MF. Enhanced synovial production of hyaluronic acid may explain rapid clinical response for high dose glucosamine in osteoarthritis. *Med Hypotheses* 1998;50:507-510.

4. Pelletier JP, Jovanovic D, Lascau-Coman V, Hilal G, Fernandes JC, Martel-Pelletier J. Relevance of animal models to clinical disease. Glucosamine sulfate reduces the structural changes in dog experimental osteoarthritis: beneficial effect through suppression of the expression of collagenase-1 [abstract]. *Symptom Modification to Structure Modification in Osteoarthritis: Focus on Glucosamine Sulfate*. ROHA Res Group, Glasgow, June 9, 1999.

5. Chapman ML, Rubin BR, Jahani M, Gracy RW. The redox state in lymphocytes from patients with rheumatoid arthritis. *J Rheumatol* 1986;13:850-852.

6. Gracy RW, Talent JM, Kong Y, Conrad CC. Reactive oxygen species: the unavoidable environmental insult? *Mutat Res* 1999;428:17-22.

7. Sato H, Takahashi T, Ide H, Fukushima T, Tabata M, Sekine F, et al. Antioxidant activity of synovial fluid, hyaluronic acid, and two subcomponents of hyaluronic acid. *Arthritis Rheum* 1988;31:63-71.

8. daCamara CC, Dowless GV. Glucosamine sulfate for osteoarthritis. *Ann Pharmacother* 1998;32:580-587.

9. Leffer CT, Phillippi AF, Leffer SG, Mosure JC, Kim PD. Glucosamine, chondroitin and manganese ascorbate for degenerative joint disease of the knee or low back: a randomized, double-blind placebo-controlled pilot study. *Milit Med* 1999;164:85-91.

10. Qui GX, Gao SN, Giacovelli G, Rovati L, Setwnikar I. Efficacy and safety of glucosamine sulfate versus ibuprofen in patients with knee osteoarthritis. *Arzneimittelforschung* 1998;48:469-474.

11. Reginster JY. Structure modification, preliminary results of a randomized placebo-controlled, double blind, three-year trial with glucosamine sulfate [abstract]. ROHA Res Group, Glasgow, June 9, 1999.

12. Barclay TS, Tsourounis C, McCart GM. Glucosamine. *Ann Pharmacother* 1998;32:574-579.

13. Setnikar I, Giachetti C, Sanolo G. Absorption, distribution and excretion of radioactivity after a single intravenous or oral administration of [14C] glucosamine to the rat. *Pharmatherapeutica* 1984;3:538-550.

14. Setnikar I, Giachetti C, Sanolo G. Pharmacokinetics of glucosamine in the dog and in man. *Arzneim-Forsch Drug Res* 1986;36:729-735.

15. Setnikar I, Palumbo R, Canali S, Zanalo G. Pharmakokinetics of glucosamine in man. *Arzneim Forsh Drug Res* 1993;43:1109-1113.

16. Talent JM, Gracy RW. Pilot study of oral polymeric N-acetylglucosamine as a potential treatment for patients with osteoarthritis. *Clin Ther* 1996;18:1184-1190.

17. Rubin BR, Talent J, Pertusi R, Forman M, Gracy R. Oral polymeric N-acetyl-D-glucosamine and osteoarthritis. In: Muzzarelli RAA, ed. *Chitosan Per Os: From Dietary Supplement to Drug Carrier*. Grottammare, Italy: Atec; 2000.

18. Caterson B, Mahmoodian F, Sorrell J, Hardingham T, Bayless M, Carney S, et al. Modulation of native chondroitin sulfate structure in tissue development and disease. *J Cell Sci* 1990;97:411-417.

19. Illum L. Chitosan and its use as a pharmaceutical excipient. *Pharm Res* 1998;15:1326-1331.

20. Felt O, Buri P, Gurny R. Chitosan: a unique polysaccharide for drug delivery. *Drug Dev Industrial Pharm* 1998;24:979-993.

21. Muzzarelli RA. Human enzymatic activities related to the therapeutic administration of chitin derivatives. *Cell Mol Life Sci* 1997;53:131-140.