Glucosamine Dose/Concentration-Effect Correlation in the Rat with Adjuvant Arthritis

ALI AGHAZADEH-HABASHI, M.H. GILZAD KOHAN, WAHEED ASGHAR, FAKHREDDIN JAMALI

Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Canada

Received 4 September 2013; revised 8 November 2013; accepted 14 November 2013

Published online 20 December 2013 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jps.23819

ABSTRACT: There is a debate on the dose dependency, concentration-effect, hence, the beneficial effect of glucosamine (GlcN), a widely used anti-inflammatory natural product. We investigated dose/concentration-effect relationship and determined its minimum effective dose/concentration in rats with adjuvant arthritis (AA, Mycobacterium butyricum in squalene) both as preventive and ameliorating interventions. To control already emerged arthritis, rats received oral doses of placebo or 160 mg/kg.day^{-1} GlcN for 6 days. For prevention, rats were orally administered 0, 20, 40, 80, or 160 mg/kg.day^{-1} GlcN commencing on the day of adjuvant injection. The arthritis index (AI), serum nitrite, and body weight were recorded. Subsequently, animals were cannulated in the right jugular veins and blood samples were collected for the determination of GlcN. GlcN ameliorated and, dose-dependently, prevented AA. It also controlled nitrite. AI was inversely correlated with GlcN dose, maximum plasma concentration, and the area under the concentration curve. Minimum effective dose was approximately 40 mg/kg.day^{-1} that correspond to maximum plasma concentration of 1.37 ± 0.24 mg/L, close to 1.6 mg/L reported for pharmaceutical grades of GlcN to humans. GlcN efficacy is dose and concentration dependent. If the data extrapolated to humans, a higher than the commonly tested 1500 mg/kg dosage regimen may provide more clear treatment outcomes. © 2013 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 103:760–767, 2014

Keywords: adjuvant arthritis; rat; minimum effective dose; dose-effect correlation; pharmacokinetics; drug effects

INTRODUCTION

Rheumatic diseases such as rheumatoid arthritis (RA) and osteoarthritis (OA) have multivariate and complex etiology and pathogenesis; hence, the identification of efficient therapies has deemed difficult. Various nonpharmacological approaches such as physical, occupational, and nutritional therapy are suggested for RA[1] and OA.[2] These approaches, however, appear to have little or no effect on the progression of the disease. Pharmacological approaches in the treatment of RA include non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, disease-modifying antirheumatic drugs, and biologics. Recently, cardiovascular complications have resulted in withdrawal of rofecoxib and valdecoxib, and have raised further questions as to the safety of the entire NSAID class.[3] Consequently, the quest for new alternative approaches for potentially effective and safe treatment of RA and OA seems imperative. Glucosamine (GlcN) is an amino monosaccharide found in the proteoglycan molecules that contribute to the strength, flexibility, and elasticity of connective and cartilage tissues.[4] In Europe, GlcN is considered as a drug but in North America it is available as a nutriceutical. Similar to a disease-modifying antirheumatic drug, GlcN appears to inhibit or halt the underlying immune process and prevent long-term damages. GlcN has been shown pharmacological effect against progression of RA[5,6] and OA[7,8] in human and experimentally induced arthritis in rats.[4] Nevertheless, even though, many animal studies confirmed potent anti-inflammatory actions for GlcN, human clinical trials have demonstrated controversial results ranging from strong effectiveness to marginal or negligible potency.[9] The explanations for this controversy include the inconsistency of the active ingredient in the commercially available GlcN products regardless of the salt used[9,10] and low plasma concentration attainable after using regular human dose of 1500 mg/day. Nevertheless, the popularity of GlcN in the treatment of joint diseases has resulted in over $2 billion global sale per year since 2008.[11,12]

The animal data demonstrating pharmacological property of GlcN are mostly generated using high doses so that the minimum effective dose and consequently minimum effective concentration is still unknown.[4] Therefore, there is a disparity between human plasma GlcN concentration and that attributed to the effectiveness in animal studies. The interstudy variability of current human pharmacokinetic data is high.[9] For example, there is over sixfold difference on reported maximum plasma concentration between the pharmaceutical grade product available in Europe (3.36 mg/L)[13] and the formulation used in a more recent clinical trial (0.492 mg/L)[14] following the same dosage regimen. This mater raises the question that whether bioavailability of GlcN form different formulations is responsible for the discrepancy between the outcomes of various clinical trials, or patients are underdosed when treated with the common 1500 mg/day regimen. Surprisingly, after many clinical trials, which reported no or marginal effects for 1500 mg/day GlcN in OA, the subsequent studies[15–19] still have used the same regimen with no attempt to try a greater daily dose. To the best of our knowledge, no information is available as to the GlcN minimum effective dose or concentration in animals or human. Herein, we report the GlcN dose/concentration-effect relationship in adjuvant arthritis (AA).

To study the pathogenic processes of arthritis, rodent models of RA and OA are useful tools. Among these animal models, we

Correspondence to: Fakhreddin Jamali (Telephone: +780-492-2807; Fax: 780-492-1217; E-mail: fjamali@ualberta.ca)

None of the authors has any conflict of interest pertaining to the content of the manuscript.

Journal of Pharmaceutical Sciences, Vol. 103, 760–767 (2014)

used AA, an experimental model of polyarthritis that has been widely used for testing of antiarthritic agents.[20] RA as a multisystem disease with underlying immune mechanism and OA as a debilitating, progressive disease are different from each other. However, several studies have focused on the possibility of correlation of cytokine levels in RA and OA with some common clinical pathological features.[21] In fact, the development of RA and OA is associated with the alteration of plasma level of some cytokines, such as Il-1β, Il-6, and TNF-α.[21] Furthermore, AA shows several common features with both OA and RA.[20,22]

MATERIALS AND METHOD

Chemicals and Reagents

Glucosamine HCl, mannosamine HCl, amantadine HCl, and fluorenylmethyloxycarbonyl chloride (FMOC-CL) were purchased from Sigma–Aldrich Canada, LTD, (Oakville, Ontario, Canada). High-performance liquid chromatography (HPLC) grade acetonitrile and water were obtained from Caledon Laboratories Ltd., (Ontario, Canada).

Animals

The study protocol was in accordance with the approved protocols of the Health Sciences Animal Care and Use Committee and University of Alberta policy. Adult healthy male Sprague–Dawley rats (230–250 g, approximately 2-month old) were obtained from the Health Sciences Laboratory Animal Services. Animals had free access to food and water and were housed under standard temperature, ventilation, and hygienic conditions.

Induction and Assessment of Experimental AA

Animals were anaesthetized with isoflurane/oxygen and injected at the tail base with adjuvant, 0.2 mL of a 50 mg/mL Mycobacterium butyricum in squalene solution (Difco Laboratories, Detroit, Michigan). Subsequently, changes in the joint and paws diameter were measured daily using a caliper with 25 μm sensitivity (Mitutoyo Canada Inc., Toronto, Ontario, Canada). The paw volume was monitored daily using the water replacement method.[23] The daily percent change in the body weight was recorded using a regular animal balance. The serum nitrite/nitrate concentrations were quantified in samples collected immediately after jugular vein insertion using the Griess reaction.[24]

The progression of AA was monitored daily by assigning an arthritis index (AI) using a macroscopic scoring system as described before[25]: for each hindpaw on a 0–4 scale, 0, no sign; 1, involvement of single joint; 2, involvement of greater than 1 joint and/or ankle; 3, involvement of several joints and ankle with moderate swelling; or 4, involvement of several joints and ankle with severe swelling. For each forepaw on a 0–3 scale, 0, no sign; 1, involvement of single joint; 2, involvement of greater than 1 joint and/or wrist; or 3, involvement of wrist and joints with moderate-to-severe swelling. AI for each rat was calculated by adding the scores for each individual paw. A maximum score of 14 could, thus, be obtained. An AI score of ≥5 was considered as the significant emergence of the disease and in the preventive experiment, animals were euthanized after reaching this score. During the treatment regimens, the experiment continued regardless of the AI score.

GlcN Treatments

Ameliorating Regimen

Animals were injected with the adjuvant, and following the emergence of AA (AI ≥ 5), they were randomly assigned into two groups (n = 3/group) of inflamed (INF)–placebo and INF–GlcN and received once daily dose of water or GlcN (160 mg/kg) for 6 days.

Preventive Regimens

Rats were divided into two main groups, healthy controls (n = 4) and INF. On day 1, INF groups were injected adjuvant before dividing them to five subgroups (n = 3–4/group): INF-0, INF-20, INF-40, INF-80, and INF-160 that were administered once daily through a gastric gavage with oral doses of water, 20, 40, 80, and 160 mg/kg GlcN commencing on day 1 for up to 16 days. To the healthy controls, only saline was injected instead of the adjuvant. AI was measured daily and the values recorded on day 16 were used for analysis.

To assess the reversibility of the effect of GlcN, on day 16 when animals in group INF-160 were still showing minimal or no sign of arthritis (AI, 0–1), the GlcN regimen was discontinued for 4 days to allow the signs of arthritis to emerge (AI, 3–5). Subsequently, the group was administered 300 mg/kg.day^{-1} GlcN for 4 days when the AI was reduced to 0–1. At that point, the group was subjected to a pharmacokinetics experiment as described under *"Pharmacokinetic Study."*

Pharmacokinetic Study

The aims of these experiments were to study the effect of inflammation on GlcN pharmacokinetics and its linearity over the range of the examined doses and, additionally, to evaluate the correlation of GlcN plasma concentration with its pharmacological effect. Control-healthy, INF-40 and INF-80 groups (on day 16), and animals that were treated with 300 mg/kg GlcN (on day 24) (see "*Preventive Regimens*") were used. They were anaesthetized with oxygen/isoflurane and cannulated in the right jugular vein.[26] After an overnight recovery, the last doses of 40, 80, or 300 mg/kg were orally administered to the AA groups. Control group received 80 mg/kg GlcN. Serial blood samples were collected, plasma separated, and stored in a −20°C freezer until assayed for GlcN.

GlcN Assay

Glucosamine plasma concentrations were determined using a precolumn derivatization reversed-phase HPLC method previously reported by our laboratory.[27] Briefly, 100 μL of rat plasma samples were spiked with 50 μL of 10 μg/mL mannosamine as an internal standard. Plasma proteins were precipitated by adding 200 μL acetonitrile followed by 1 min vortex mixing and centrifugation for 3 min at 10,000g. In a test tube, 100 μL of the supernatant was added and 50 μL of borate buffer (0.2 M, pH 8.5) was added followed by 50 μL of freshly prepared FMOC-CL as derivatizing agent. The mixture was shaken for 1 min and incubated for drivetization in a water bath at 30°C for 30 min. Subsequently, 50 μL of amantadine HCl was added and samples were mixed and diluted with 1 mL acetonitrile–water (1:1). Five microliter of the final solution was then injected onto the HPLC system (Shimadzu prominence, Mandel Scientific, Guelph, Ontario, Canada). The chromatographic separation was achieved on Phenomenex C18 (100 × 4.6 mm^2, i.d. 3 μm)