PHARMACOKINETIC PROFILE OF GLUCOSAMINE AND CHONDROITIN SULFATE ASSOCIATION IN HEALTHY MALE INDIVIDUALS

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SUMMARY

Osteoarthrosis is a chronic joint disease that, once patent, leads to a progressive functional disability. As proteochondroitin sulfates are the major contents of the cartilage, it is expected that the ingestion of glucosamine and chondroitin might improve the biological status of that tissue. As we could not find any studies on the pharmacokinetic profile of this association by oral administration route in human beings, the objective of this study was to evaluate it by using the association of glucosamine sulfate (GS) and chondroitin sulfate (CS) given to two groups of twelve healthy male volunteers (group I: one capsule containing 500 mg of GS and 400 mg of CS; group II: four capsules with the same content). Blood samples were collected at pre-determined time intervals up to 48 hours post-dosing. GS and CS were measured in plasma by the DMMB (1,9,dimethyl-dimethilene blue) method. Maximum concentration was achieved within 2 hours (average ± SE; 0.893±0.093 μg/ml, group I; and 2.222±0.313 μg/ml, group II). Areas under curve up to 48 hours were 10.803±0.965 μg-hr/ml and 38.776±2.981 μg-hr/ml for groups I and II, respectively. Both groups showed a second peak after 18 hours, indicating an enterohepatic flow. Our results indicate that this association is absorbed through the oral route by a saturable mechanism, which can enable its use in clinical treatments.

Keywords: *Osteoarthrosis; Chondroitin Sulfate; Glucosamine; Proteoglycans*

INTRODUCTION

Osteoarthritis is a chronic disease of the joints that causes patients to suffer a progressive functional disability. This disease is not age-related, but once patent, it progresses with aging[1-3]. It is related to a loss of joint functional ability, and is characterized by continuous pain. The lack of cure for osteoarthritis results in pain being the only issue treated by means of topical agents, anti-inflammatory agents, opioids and a-adrenergic analgesics, intra-articular agents, such as depocorticosteroids, hyaluronate and beta-irradiating radionucleotides, sulfated glycosaminoglycans, such as the chondroitin sulfate (CS) and glucosamine sulfate (GS)[4-6]. On the other hand, surgical treatment by means of debridements, osteotomies and total prosthesis have been providing good effects.

Glucosamine (N-acetyl-glucosamine) is a byproduct of the glucose metabolism and this degradation byproduct is one of the contents of the gallate and glycosaminoglycans[6]. Glycosaminoglycans are linear polymers formed by repeated disaccaridic units where one of the units is invariably a hexosAmine (D-glucosamine or D-galactosamine) and the other is an hexuronic acid (glucuronic or iduronic) or a non-ramified sequenced galactose presenting replacements of sulfate groups in many positions of the polysaccharidic chain[7]. The three disaccharidic units: N-acetyl-galactosamine-hexuronic acid; N-acetyl-glucosamine-hexuronic acid, and N-acetyl-glucosamine-galactose represent the basic units in the formation of glycosaminoglycans chain. The galactosaminoglycans, of which hexosamine will always be a D-galactosamine, are formed by the chondroitin sulfate family; and glycosaminoglycans, of which hexosamine will always be a D-glucosAmine, are formed by hyaluronic acid, heparan sulfate, heparin and cheratam sulfate.

In the category of chondroitins, we will find non-sulfated chondroitins, 4-sulfated, 6-sulfated and the dermatan sulfate[1].

Chondroitin sulfates are formed by repeated disaccharidic units of glucoronic acid bonded by a N-acetyl-galactosamine, and present an ether sulfate at 4- or 6- position and for this reason the polysaccharide formed is named chondroitin 4-sulfate or 6-sulfate.

Chondroitin sulfate chains vary in their average length from one tissue to another or within the same tissue. In general, the average molecular mass of the chondroitin sulfate chains in the joint cartilage is reduced with age [8]. Except for hyaluronic acid, all glycosaminoglycans and/ or galactosaminoglycans are found as proteoglycans.

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The kinds of proteoglycans in cartilage present with different hydrodynamic volumes, being this fact related to the number of glycosaminoglycan chains. It is important to remember that most of the glycosaminoglycans found in bones and cartilages are constituted by chondroitin sulfates.

Proteoglycans (PGs) are complex macromolecules containing a protein structure with one or more glycosaminoglycan chains covalently bonded[9-13]. The protein structure of proteoglycans is centrally located in the molecule and is often supported by a "bed" formed by glycosaminoglycan chains.

In cartilages, the chondrocytes synthesize the various kinds of proteoglycans, which, together with the collagen matrix, form a supramolecular complex giving to this tissue a biological spring function, able to resist to high compressive forces, in addition of being involved in tissue growth and remodeling. Cartilages have a very high concentration of proteoglycans, which are responsible for its morphological structure and for the nourishment of cartilaginous cells.

As proteochondroitin sulfates are the major contents of cartilage, it is expected that, by providing glucosamine and chondroitin, tissue's biological conditions are improved. However, there are controversies surrounding the proposal of treatment benefits with glucosamine and chondroitin in patients with osteoarthritis[14-17]. The present study shows that the administration of glucosamine sulfate associated to chondroitin sulfate is orally absorbed by healthy individuals.

CASE SERIES, MATERIALS AND METHODS

Case Series

This protocol has been conducted according to the Declaration of Helsinki, described in the Resolutions 196/96 and 251/97 of the CNS/MS – Guidelines for Researches Involving Human Beings. The experimental protocol and the free and informed consent term (FICT) have been approved by the Committee of Ethics in Research at the Hospital do Rim e Hipertensão, certified by CONEP/Ministry of Health. Male healthy volunteers, with ages ranging 18-45 years old and body mass index (BMI) between 19 and 25 have been included in the study. Individuals with clinically significant abnormalities in biochemical, blood and Urine I tests, presenting gastrointestinal, cardiovascular, hepatic, hematopoietic, renal, or respiratory diseases, or with allergy history to CS and GS have been excluded from the study. Other exclusion criteria included: recent exposure (less than 3 months) to experimental or other drugs, recent intake or history of alcohol or illegal drugs abuse, smokers, recent blood donations (less than 3 months), HIV, Hepatitis B and C positive. The study was conducted at the Clinical Pharmacology Unit of the Hospital do Rim e Hipertensão, according to the rules of the Good Clinical Practices and the FICT was signed by all volunteers prior to the study.

Study Design

This was an open-label, randomized, single-dose and single-period study. Twenty four volunteers were randomly assigned to two groups of 12 individuals each. Group I (GI) received one 500-mg capsule of CS and 400 mg of GS; Group II (GII) received four capsules. Volunteers were hospitalized the day before drug administration. Administration was provided at 7AM, after 10 hours of fasting. Volunteers remained seated during the four subsequent hours. Standardized meals were served within 4, 7, 11, 13, 24, 28, 31, 35, 37, and 48 hours after drug administration.

Samples collection and handling

Blood samples of the volunteers in this study were collected before drug administration (baseline collection) and within 0.5, 1, 2, 4, 5, 6, 8, 10, 12, 18, 24, 36, 48, and 168 hours in Vacuntainer® tubes, totaling 15 aliquots. EDTA was used as anticoagulant. Samples were collected during the hospitalization period within 2 minutes of scheduled time, maintained in ice and centrifuged (10 minutes at 720 x g, 4°C). Isolated plasma was frozen in two (2) aliquots and maintained at -20°C until analysis. The time between blood collection and plasma freezing did not exceed 30 minutes.

Analytical method

The GS and the CS were dosed by the method using 1,9,dimethyl-dimethylene blue[18] (DMMB), developed for determining sulfated glycosaminoglycans. The method, validated according to ANVISA's guidelines, is based on the liquid-liquid extraction and spectrophotometry quantification. The method is linear between 0.5 and 5.0 μg/mL. Both GS and CS are stable in plasma within assay circumstances, at three cycles of freezing/unfreezing, to light, and up to three months of storage at –20°C. The intra-assay accuracy ranges from 3.1 to 9.0% and inter-assay from 2.9 to 13.9%; intra-assay accuracy ranges from 90 to 110% and inter-assay, from 100 to 115%.

Glucosamine and Chondroitin extraction

One milligram of papain dissolved in phosphate:cysteine buffer at a pH of 6.5 containing EDTA 0,10 M was added to each 2-ml aliquot of plasma sample. Samples were incubated at 55° C for 16 hours. After incubation, 500 μL of NaCL 4 M were added, and, after agitation, an amount equivalent to 10% of the total volume of 90% trichloracetic acid (TCA). The supernatant was precipitated with absolute ethanol (3 times its volume) for 16 h at –20° C. The precipitate was vacuum-dried and re-suspended in 100 μL of water. One milliliter of the DMMB reagent was added to the water-diluted samples, followed by a careful agitation. Reading was performed in a spectrophotometer at 525 nm, within 3 minutes counting from the addition of DMMB. The standard curve was performed using the chondroitin sulfate (CS) solution (Aché Laboratórios Farmacêuticos).

	Group I				Group II			
	Age (yrs)	Weight (kg)	Height (m)	BMI (kg/m^2)	Age (yrs)	Weight (kg)	Height (m)	BMI (kg/m^2)
average:	26	67.2	1.74	22.12	29	67.4	1.73	22.49
SD:	7	7.1	0.04	1.81	9	10.0	0.08	2.10
SE:	2	2.7	0.20	1.35	3	3.2	0.29	1.45
minimum:	19	59.4	1.68	19.85	20	52.4	1.62	19.29
median:	24	65.9	1.74	21.49	28	65.2	1.72	22.54
maximum:	45	84.7	1.84	25.00	45	86.5	1.86	25.00

Table 1 - *Anthropometrical data of the volunteers*

RESULTS AND DISCUSSION

Case Series

Anthropometrical data of the healthy volunteers participating in the study are summarized on Table 1. We can see that the study population is homogeneous and both groups are similar.

Clinical Assay

The volunteers were observed during hospitalization and specifically inquired about adverse events at each 4 hours. During this period, no adverse event was observed. After 7 days of hospital discharging, volunteers were back for biochemical tests, ECG and clinical examination, when they were discharged from study.

Plasmatic kinetics

Figure 1A shows the average ± SE of the gross results for both

groups (including endogenous concentration). After oral administration, plasmatic concentration is increased, reaching maximum values after 2 hours ($T_{máx}$), decreasing at its minimum value after 8 hours from intake, both for group I and group II. A second peak happens after 18 hours from intake, probably due to the enterohepatic re-flow[19]. Figure 1B shows the average ± SE of the net result, that is, after subtracting baseline concentration (endogenous).

The C_{max} seen (above baseline concentration) was 0.893± 0.093 μg/mL and 2.222±0.313 μg/mL and the AUC0-48h 10.803±0.965 μg-h/mL and 28.543±6.704 μg-h/mL (average ±SE), respectively for GI and GII. After 18 hours from intake, concentration was 0.365±0.041 μg/mL and 0.974±0.198 μg/mL, for GI and GII, respectively. Table 2 summarizes the average ± SE of the computed parameters disregarding the baseline plasmatic concentration (endogenous).

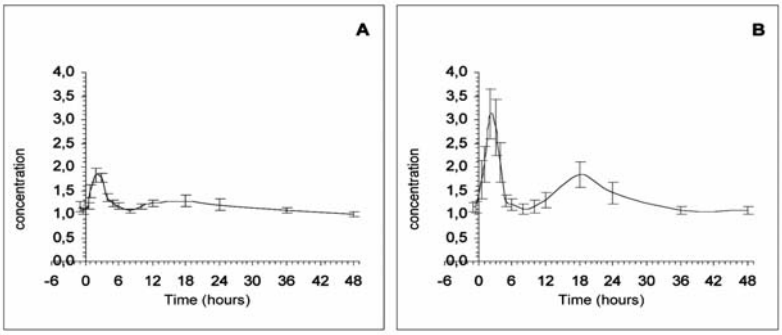

Figure 1 - *Plasmatic kinetics of the association of 400-mg glucosamine sulfate and 500-mg chondroitin sulfate capsules. The points represent the average ± SE of twelve (12) volunteers. A, group I (1 capsule); B. group II (4 capsules).*

	GI	GII	GII/GI
(CS) dosage (μg/kg)	5952.4±628.9	23738.9±3522.1	4.0
(GS) dosage (μg/kg)	7440.5±786.1	29673.6±4402.6	4.0
C_{max} (μg/mL)	0.893±0.093	2.222±0.313	2.4
AUC_{0-t} (μg-hr/mL)	10.803±0.965	28.543±6.704	2.6
AUC_{0-inf} (μg-hr/mL)	12.000±1.072	38.776±2.981	3.2
C_{18h} (μg/mL)	0.365±0.041	0.974±0.198	2.7
half-life (h)	16.931±1.902	25.515±2.560	1.5
C_{18}/C_{max}	0.41	0.44	1.0

Table 2 - *Pharmacokinetic parameters calculated by subtracting baseline concentration (endogenous). Data represent the average ± SE for each group. CS, chondroitin sulfate; GS, glucosamine sulfate. GI, group 1, one capsule administered; GII, group 2, four capsules administered.*

Absorption kinetics for CS and GS is not linear, as shown by the ratios between C_{max} and AUC0-48h (2.389 and 2.642, respectively, Table 2). This ratio is maintained in the 18-hour peak, resulting from enterohepatic re-flow (2.668), which reinforces the evidence that the absorption phase is limiting. Furthermore, half-life is also dosage-dependent (16.931±1.902 h and 25.515±2.560 h, for 1 and 4 capsules, respectively).

CONCLUSION

Our results evidence that a single dose of up to four capsules of 500 mg CS associated with 400 mg GS is well tolerated and the profile found is consistent to a 12-hour administration.

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