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Oral bioavailability of chondroitin sulfate (Condrosulf®) and its constituents in healthy male volunteers

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Summary

Objective: Drug treatment of osteoarthritis (OA) includes symptomatic slow-acting drugs (SYSADOA). This class of compounds have a slow onset of action and improve OA symptoms. Among the SYSADOA, Condrosulf® (manufactured by IBSA), whose active ingredient is chondroitin sulfate, has proven to be a valuable therapeutic tool for the symptomatic treatment of OA after oral administration. The aim of this study was to assess the bioavailability of chondroitin sulfate and its constituents after oral administration of Condrosulf® to 20 healthy male volunteers. Pharmacokinetic parameters and the structure and properties of plasma chondroitin sulfate were determined after administration of Condrosulf®. The possible physiological regulation of plasma levels of endogenous chondroitin sulfate during the day was also assessed.

Design: Condrosulf® (composed of bovine origin chondroitin sulfate, 4 g) was orally administered to 20 healthy human volunteers, and chondroitin sulfate derivatives were extracted and purified from plasma over a 48 h period. Polysaccharide fractions absorbed by oral route were characterized and quantified by agarose-gel electrophoretic technique, and densitometric scanning. In addition, the percentage of constituent disaccharides and charge density were measured in an effort to physico-chemically characterize chondroitin sulfate fractions absorbed per os.

Results: Plasma levels of endogenous chondroitin sulfate were detectable in all subjects, and the mean values calculated on six subjects varied during the day from 0.3 to 5.3 μg/ml. After administration of Condrosulf®, chondroitin sulfate plasma levels increased (more than 200%) in all subjects with a peak concentration after 2 h, with the increase reaching significance from 2 to 6 h. Absorption of exogenous chondroitin sulfate was also proved by the change in the composition of disaccharides in plasma after drug administration with respect to baseline. A significant decrease in the relative amount of non-sulfated disaccharide was measured (reaching the minimum relative percentage of 22.96±11.68% at 4 h). At the same time 4-sulfated disaccharide increased to a maximum of 60.50±10.45% after 4 h and 6-sulfated disaccharide appeared in blood, reaching a maximum concentration of 17.33±6.52% after 2 h. Concomitantly the mean charge density increased from 0.40±0.09 at pre-dose to a maximum of 0.78±0.11 4 h after Condrosulf® administration.
As for safety, the treatment was well tolerated and did not determine any relevant change in vital signs nor ECG.

Conclusions: From this study and literature data, it appears that exogenous chondroitin sulfate (Condrosulf®) is absorbed as a high molecular mass polysaccharide together with derivatives resulting from a partial depolymerization and/or desulfation. © 2002 OsteoArthritis Research Society International. Published by Elsevier Science Ltd. All rights reserved.

Key words: Chondroitin sulfate, Glycosaminoglycans, Oral route, Osteoarthritis.

Introduction

Chondroitin sulfate (CS) is an unbranched, polydisperse, complex glycosaminoglycan extracted and purified from various tissues, with polysaccharide chains composed mainly of disaccharide units of sequence [-N-acetyl-D-galactosamine β 1:4–D-glucuronate β 1:3-]. Depending on the source, different non-sulfated and sulfated disaccharides are present within the polysaccharide chains[1–3]. CS is a ubiquitous components of all connective tissue extracellular matrices where it serves a number of functions mainly covalently attached to proteins in the form of proteoglycans[4,5]. Due to the presence of sulfate groups in different amounts and located in various positions (2 and 3 of uronic acid[6], and 4 and 6 of N-acetyl-galactosamine residues), CS represents a very heterogeneous family of polysaccharides, in terms of degree of sulfatation, molecular mass, relative amounts of iduronic acid and glucuronate, depending on the tissue of origin.

CS exhibits a wide variety of biological functions mainly due to the presence of rare oversulfated structural building units that form domain structures that interact specifically with other molecules, such as the regulation of neuronal patterning in the retina[7], interactions with fibronectin[8], neurite outgrowth promoting activity[9], modulation the adesive function of $\alpha_4\beta_1$ integrin[10], activation of monocyte and B-cell[11], and activation of plasminogen[12].

Osteoarthritis (OA) is a common form of joint disorder in developed countries[13]. OA is heterogeneous condition with various clinical expressions and it is therefore considered a syndrome. The most common symptoms are pain and functional disability resulting from destructive changes of the osteoarthritic joint. Current treatment of OA is not aimed at a cure but at palliative management; it includes physical, pharmacological and surgical approaches. Drug treatment includes analgesics, NSAIDs, and symptomatic slow-acting drugs (SYSADOA). The latter class of compounds have a slow onset of action and improve OA symptoms after about a month of the treatment[14]. Some of them are administered orally and some intraarticularly. Among the SYSADOA,

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Condrosulf® (manufactured by IBSA), whose active ingredient is CS, has proven to be a valuable therapeutic tool for the symptomatic treatment of OA. CS is also employed as an antiinflammatory[15], chondroprotective and antirheumatic drug[16–19], and several controlled trials showed its effects as a SYSADOA with application in the therapy of OA of the knee and in articular cartilage OA[14,20–24] with very good tolerability[25,26].

CS (Condrosulf®) is mainly employed by the oral route allowing a more simple drug use compatible with long term administration[14,18,23,24]. Pharmacokinetic studies have been performed on man and animals after oral administration of a tritiated CS derivative[27,28]. The results show a significant increase of plasma levels of CS as compared with predose levels over a full 24 h period. Moreover, adsorbed CS reaches the blood compartment as high, intermediate and low molecular mass derivatives, with a very irrelevant part (less than 1–2%) of radioactivity released by exchange with water[28].

In the present study, Condrosulf® (composed of bovine CS) was orally administered to 20 healthy human volunteers, and CS derivatives were extracted and purified from plasma over a 48 h period. Polysaccharide fractions absorbed by the oral route were characterized and quantified by validated agarose-gel electrophoretic technique, and densitometric scanning. Pharmacokinetic parameters were determined and the possible physiological regulation of plasma levels of endogenous CS during the day was also assessed. In addition, the percentage of constituent disaccharides and charge density were measured in an effort to physico-chemically characterize CS fractions absorbed by the oral route.

Experimentals

SUBJECTS

A total number of 20 healthy male volunteers participated to the study. Six volunteers, randomly selected, underwent evaluation of plasma levels of endogenous CS before starting the study period. The Clinical Investigator gave his approval to the participation of each subject in the study on the basis of acceptable medical history and findings in the physical and instrumental (ECG, laboratory) examinations. Written informed consent was obtained prior to inclusion to each study period, as per protocol.

The healthy volunteers were caucasian males aged 18–30 years, within ± 15% of ideal body weight, with normal values of blood pressure (systolic blood pressure of 100–140 mmHg and diastolic blood pressure of 60–90 mmHg) and heart rate (60–80 bpm), and no clinically relevant abnormal values in the routine blood chemistries. They showed no clinically relevant electrocardiogram abnormalities, no clinically important abnormal physical findings, and no known allergy to drugs or chemicals or allergic reactions in general, which may have affected the results of the study. They also had no relevant history of skin, renal, hepatic, gastrointestinal, cardiovascular, haematological, respiratory, endocrine or central nervous system diseases (in particular no history of kidney or liver insufficiency, no gastrointestinal or bowel movement disorders). Volunteers received no medication during the first week prior to the start of the trial that might affected the validity of the study. Volunteers also stated that they received no administration of NSAIDs, salycilates and barbiturates within the last month. Furthermore, they had no participation other drug trials or blood donation during the 3 months prior to the start of the study, no history of drug, alcohol, caffeine or tobacco abuse (more than 60 g/day of alcohol, 5 cups/day of coffee or 10 cigarettes/day). None of the volunteers took any medication other than the study treatment during the study.

The volunteers had ability to comprehend the full nature and purpose of the study, to co-operate with the investigator and to comply with the requirements of the study. They gave written informed consent.

STUDY PROTOCOL

This was an open, single centre, single dose study. 10 capsules of Condrosulf® 400 mg, Batch No. 990709, expiry date July 2002, were administered as a single oral dose.

The study drugs were provided by IBSA (Institut Biochimique S. A., CH-6915 Pambio Noranco, Lugano, Svizzera) to the Clinical Centre (Cross Research S. A. Phase I Unit, CH-6864 Arzo, Svizzera) in excess of the amount necessary for the study (25% excess). The study medications were stored in a cool, safe locked place and were dispensed only by the investigator or authorized personnel. The study drug was exclusively used for the present clinical trial and was only administered to the subjects enrolled in the study. At the end of the study, all the unused supplies were returned to the sponsor, after assessment of drug accountability.

Subjects took no food or drink (apart from water) for about 12 h (i.e. overnight) before administration and for up to 2 h after treatment. Starting from 24 h before drug administration, the intake of food containing high quantities of glycosaminoglycans was kept low. The dinner of the day before each drug administration was consumed at the clinical centre. A standardized light breakfast was served at approximately 10.00 a.m. (2 h after drug administration), lunch at approximately 1.00 p.m. (after the 5th h sampling) and dinner at approximately 8.00 p.m. (after the 12th h sampling).

The volunteers were asked to avoid physical activity during the 3 days preceding the study start. The evening preceding drug administration and start of blood sampling the volunteers attended the Clinical Centre to be hospitalized.

The study periods included a single oral administration at about 8.00 a.m. of day 1, followed by a 48 h observation period. On the last day of the study period (day 3), each volunteer underwent blood and urine tests and electrocardiogram evaluation for post-study assessment. During the study period, a single administration was performed on day 1 at 8.00–8.33 a.m. Each volunteer swallowed 10 capsules of the test drug (Condrosulf® 400 mg) together with 400 ml of tap water (200 ml just before and 200 ml during administration).

PLASMA SAMPLES COLLECTION

Venous blood samples (15 ml) were taken from a vein of the forearm using an indwelling catheter. The cannula was rinsed after each sampling. Blood samples were collected in tubes containing citrate as anticoagulant at the following times: pre-dose and 0.5, 1, 2, 4, 6, 8, 12, 16, 24, 48 h after drug intake. The samples were immediately stored at 4°C, centrifuged within 1 h at 4°C for 10 min to obtain plasma and immediately divided into two 2.5 ml aliquots, transferred into pre-labeled test tubes and stored frozen at −20°C until analysed.

Endogenous CS, from six of the 20 volunteers enrolled in the study, was assessed on venous blood samples (12 ml) taken with the same procedure described above at the following times: 8, 10, 12 a.m., 2, 4, 6, 8, 10, 12 p.m for 2 consecutive days. Two 2 ml aliquots were obtained and stored frozen at −20°C until analysed. Endogenous CS was quantitatively determined by agarose-gel electrophoresis and densitometric scanning according to the analytical procedure illustrated below.

ANALYTICS

Materials

Protease type XXI from *Streptomyces griseus* [E.C. 3.4.24.31] was from Sigma. Ecteola-cellulose (condensation product of epichlorohydrin, triethanolamine and cellulose, cross-linked fibers; capacity of 0.3–0.4 meq/g, particle size of 0.05–0.2 mm) was from Serva, Heidelberg, Germany. High purity agarose and barium acetate were from BioRad. 1,2-diaminopropane and cetyltrimethylammonium bromide were from Merck, Darmstadt, Germany. 5 μm Spherisorb SAX (trimethylammoniopropyl groups $Si\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}N^+(CH_3)_3$ in Cl^- form) was from Phase Separations Limited, Deeside Industrial Park, Deeside Clwyd, U.K. Chondroitinase ABC from *Proteus vulgaris* [E.C. 4.2.2.4] was obtained from Sigma. Non-sulfated and variously sulfated unsaturated CS (ΔDi-0s, ΔDi-4s, ΔDi-6s, see abbreviations) were obtained from Seikagaku Corporation, Tokyo, Japan. All the other reagents were analytical grade.

Extraction and purification of plasma CS

Extraction of CS from plasma samples was performed according to the methods reported elsewhere[29,30], with slight modifications. 500 μl protease from *Streptomyces griseus* (10 mg/ml 50 mM Tris–Cl buffer pH 8.0) was added to 1000 μl of plasma. After incubation at 37°C for 24 h, 1000 μl 0.1 M acetic acid and 500 μl NaCl 3 M were added. The mixtures were boiled for 5 min, and then centrifuged at 5000 ***g*** for 5 min, and 1000 μl NaOH 0.1 M was added to the supernatants; two volumes of acetone were then added and solutions stored at −20°C for 24 h. The precipitates were recovered by centrifugation at 5000 ***g*** for 15 min and dried at 50°C for 12 h. The dried precipitates were dissolved in 1000 μl of distilled water by prolonged mixing and CS further purifed on anion-exchange resin (Ecteola-cellulose). After centrifugation at 10,000 ***g*** for 5 min, the supernatant was applied to a column (1 cm×2 cm) packed with about 1.5 ml of Ecteola-cellulosa previously washed with 1 M NaOH and 1 M HCl and equilibrated with 0.05 M NaCl. After washing the resin with 2 volumes of 0.05 M NaCl, 5 ml of 3 M NaCl were added. Two volumes of acetone were added to the eluate (5 ml) and stored at +4°C for 24 h. After centrifugation at 5000 ***g*** for 15 min, the pellet was dried at 60°C for 6 h.

Agarose-gel electrophoresis

Validated agarose-gel electrophoresis (see Appendix) was performed according to Volpi[31,32] in a Multiphor II electrophoretic cell (Pharmacia LKB Biotechnology). Samples were dissolved in 20 μl distilled water and layered on 0.5% agarose-gel plate and run in 0.05 M 1,2-diaminopropane buffered to pH 9.0 with acetic acid for 3 h at 60 mA. After migration, the plate was soaked in cetyltrimethylammonium bromide (0.1% solution) for about 12 h, dried and then stained with toluidine blue (0.2% in ethanol–water–acetic acid, 50:49:1 v:v:v) for 30 min and destained with ethanol–water–acetic acid 50:49:1 v:v:v.

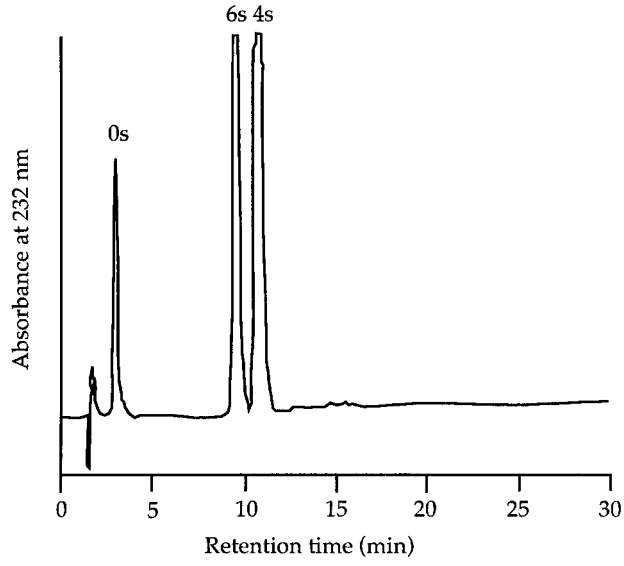

Fig. 1. SAX-HPLC chromatogram of non-sulfated (0s), 6-sulfated (6s) and 4-sulfated (4s) CS disaccharides.

Densitometric analysis was performed with a densitometric unit composed of a Macintosh IIsi computer interfaced to Microtek Color Scanner from Microtek International Inc., Hsinchu, Taiwan. The IMAGE processing and analysis program, Version 1.41 from Jet Propulsion Lab., NASA, Florida, U.S.A. was used for densitometric analysis of agarose-gel bands.

Disaccharide composition of endogenous CS and CS after oral administration of Condrosulf®

Samples dissolved in 40 μl of 50 mM pH 8.0 tris-HCl buffer were incubated with 50 munits of chondroitinase ABC. The reactions were stopped after 3 h incubation at 37°C, by boiling for 1 min. The constituent disaccharides were determined by SAX-HPLC at 232 nm[33,34]. Isocratic separation was from run 0 to 5 min with 0.1 M NaCl, pH 4.00; linear gradient separation from 5 to 30 min with 100% 0.1 M NaCl, pH 4.00 to 50% 1.2 M NaCl, pH 4.00. Flow rate was 1.4 ml/min. Separation of unsaturated non-sulfated and variously sulfated disaccharides produced by the action of the bacterial lyase was performed using standards supplied by Seikagaku Kogyo Co. (see Fig. 1).

The following parameters were provided for each time after oral administration of Condrosulf®: relative percentage of each unsaturated disaccharide calculated per 500 μl of plasma, namely ΔDi-0s (non-sulfated disaccharide), ΔDi-6s (6-monosulfated disaccharide), ΔDi-4s (4-monosulfated disaccharide). Therefore, charge density (sulfate to disaccharide ratio) of CS was calculated.

PHARMACOKINETIC PARAMETERS[35–37]

The percentage increase in CS with respect to baseline value was calculated at each time point after drug administration. The following pharmacokinetic parameters were

obtained from the individual values of CS plasma concentrations (μg/ml) vs time (hours), determined up to 48 h after Condrosulf® administration using the validated software KINETICA™ 2000 version 3.0, InnaPhase Corporation, Philadelphia, U.S.A. (KINETICA 2000 User's Manual. Innaphase Corporation. Philadelphia, U.S.A., 1999):

C_{max}. The highest concentration value found in plasma.
t_{max}. The time from administration at which the C_{max} value is found.
AUC_{0-48}. The area under the plasma concentration vs time curve up to the last sampling time calculated by the linear–linear trapezoidal rule.
AUC_{0-24}. The area under the plasma concentration vs time curve up to the 24 h sampling time calculated by the linear-linear trapezoidal rule.

Calculation of AUC_{∞}, K_{el}, $t_{1/2}$ and MRT was not feasible because biased by the presence of the endogenous compound.

For endogenous CS levels, the following parameters were calculated: C_{max}, t_{max}, AUC_{0-24} and AUC_{0-48}. Moreover, for each subject the mean of the values measured from 0 to 16 h over two subsequent days was calculated.

STATISTICAL ANALYSIS

The data documented in this trial and the parameters measured were compared and evaluated using classic statistics: mean, standard deviation, coefficient of variation (%), minimum and maximum values (for quantitative variables) and by frequencies (qualitative variables).

Descriptive statistics were performed by means of either SAS® version 8e for Windows® (SAS/STAT® User's Guide, Version 6, Fourth Edition, SAS Institute Inc., Cary, NC, U.S.A., 1990) or Kinetica™.

The CS plasma concentrations, μg/ml plasma CS for each time, measured after drug treatment were compared to basal values by means of a *t*-test. The same comparison was made for endogenous CS levels by comparing mean CS concentrations at different sampling times from 2 to 16 h to time 0 levels. The ANOVA test was then applied to compare within group the increase/decrease at the different times for treated and endogenous CS. When significant overall differences were found, the Student–Newman–Keuls test was applied to identify significantly different means.

The above statistical comparisons of the significant pharmacokinetic parameters obtained for test vs reference treatment were made using KINETICA™.

ETHICS AND LEGAL CONSIDERATIONS

The study was performed in accord with the relevant guidelines of the Declaration of Helsinki, 1964, as amended in Tokyo, 1975, Venice, 1983, Hong Kong, 1989 and Somerset West, 1996.

Approval of the study protocol by the relevant local (Canton Ticino) Research Ethics Committee was obtained before the start of each study period. Federal Authorities were informed about the study.

The present Clinical Trial was carried out according to the general principles of 'ICH Topic E6, CPMP/ICH/135/95', July 1996 including post Step 4 errata, status September 1997.

Table I
Demographic data of enrolled volunteers

Subject	Age (y)	Weight (kg)	Height (cm)
01	25	61.2	177
02	28	72.4	182
03	25	69.1	178
04	22	75.1	179
05	29	64.2	169
06	26	64.8	178
07	24	76.0	187
08	25	64.2	169
09	24	69.5	181
10*	27	95.3	190
11*	25	76.2	181
12	24	62.2	181
13*	25	80.0	180
14	26	70.3	171
15	21	66.3	176
16*	22	62.5	183
17*	21	68.2	180
18	21	66.3	180
19	25	77.0	174
20*	28	77.7	175

*Indicates the subgroups of volunteers evaluated for endogenous levels of CS.

Table II
Mean endogenous CS plasma pharmacokinetic parameters

	C_{max} (μg/ml)	t_{max} (h)	AUC_{0-48} (μg×h/ml)	AUC_{0-24} (μg×h/ml)
Mean	3.88	21.7	110.5	49.2
S.D.	0.85	14.1	22.1	16.5
CV%	21.9	64.9	20.0	33.5
Min	3.2	16.0	88.5	22.10
Max	5.3	24.0	152.0	71.1
N	6	6	6	6

Results

DEMOGRAPHIC DATA

Demographic data of the 20 subjects enrolled in the study were: age (years) 25.2±2.1, weight (kg) 71.1±5.6, height (cm) 178.1±4.7. Six among these volunteers participated in the study for evaluation of endogenous levels of CS. No differences were found comparing this subgroup with the total group (Table I).

The treatment was well tolerated and did not cause relevant changes in vital signs or ECG.

QUALITATIVE AND QUANTITATIVE DETERMINATION OF CS

Agarose-gel electrophoresis permits qualitative and quantitative determination of CS after extraction from plasma (see Appendix for method validation). CS was qualitatively evaluated by its migration time vs standard. For example, Fig. 2 illustrates agarose-gel electrophoresis of CS extracted and purified from 1 ml plasma of subject 1 at different times (from 0 to 48 h) after oral administration of 4 g of Condrosulf®.

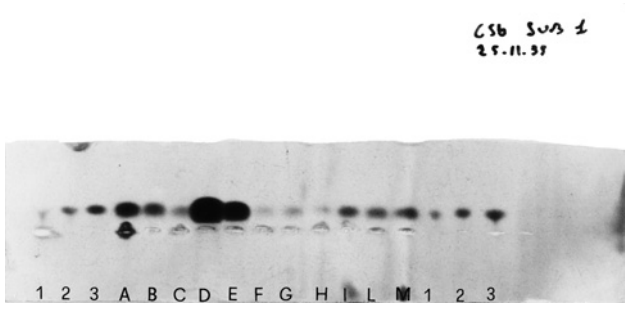

Fig. 2. Agarose-gel electrophoresis of endogenous chondroitin sulfate (Predose) and chondroitin sulfate extracted and purified from Subject 1 plasma after administration of Condrosulf® at various times. 1: 1 μg condroitin sulfate. 2: 3 μg condroitin sulfate. 3: 5 μg condroitin sulfate. (a) Pre-dose. (b) 0.5 h. (c) 1 h. (d) 2 h. (e) 4 h. (f) 6 h. (g) 8 h. (h) 12 h. (i) 16 h. (l) 24 h. (m) 48 h.

PRE-DOSE AND BASAL CS PLASMA CONCENTRATIONS

Basal CS concentrations are shown in Fig. 3. Plasma levels of endogenous CS were detectable in all the subjects (six volunteers, see Table I) at any time of the day and ranged from 0.3 to 5.3 μg/ml plasma. The average endogenous CS plasma concentration at each time of the day was then calculated from the values obtained at the same time of the day in the two consecutive days for each volunteer. The means on the 6 subjects for each time, from 0 to 48 h, varied during the day from a minimum average value of 1.53 μg/ml at 16 h to a maximum average amount of 3.37 μg/ml at 24 h (Fig. 3).

Pre-dose plasma levels of CS were detectable in all the subjects and averaged 3.80±1.77 μg/ml (ranging from 1.4 to 7.6 μg/ml).

ORAL ADMINISTRATION OF CONDROSULF®

After administration of Condrosulf®, CS plasma levels increased in all the subjects with a peak concentration at 2 h (Fig. 3), except for subject 10, who showed a peak at 4 h, and subject 2 at 8 h. Percentage increase and significance (*t*-test) with respect to basal value were calculated. The increase in CS plasma levels was significant from 2 to 6 h after drug administration. In particular, 2 and 4 h after administration CS plasma levels increased by more than 200% with respect to pre-dose endogenous CS (Fig. 3); 48 h after drug administration CS plasma levels were detectable in all the subjects and averaged 2.86±1.56 μg/ml.

Mean CS pharmacokinetic parameters under basal conditions and after administration of drug are shown in Tables II and III. The AUC_{0-48} averaged 110.5±22.1 μg×h/ml. The tract from 24 to 48 h is likely to represent endogenous CS levels because there is no important decrease in CS concentration after 24 h. The AUC_{0-48} was 179.1±47.9 μg×h/ml after administration of Condrosulf®. Under basal conditions the AUC_{0-24} averaged 49.2±16.5 μg×h/ml. In order to factor out the contribution of basal CS in the calculation of AUC, the AUC_{0-24} was calculated; it averaged 111.4±28.2 μg×h/ml for Condrosulf®. Under basal conditions the C_{max} averaged 3.88±0.85 μg/ml and t_{max} 21.7±14.1 h. The maximum measured plasma concentration was 12.73±4.69 μg/ml after administration of Condrosulf® with a t_{max} of 2.4±1.4 h. In order to further remove the influence of endogenous CS levels in the calculation of AUC, the area under the minimum concentration vs. time curve up to the 24 h sampling time was calculated for each subject and subtracted from the AUC_{0-24}. The $AUC_{(0-24)min}$ averaged 65.2±29.1 μg×h/ml after administration of Condrosulf®.

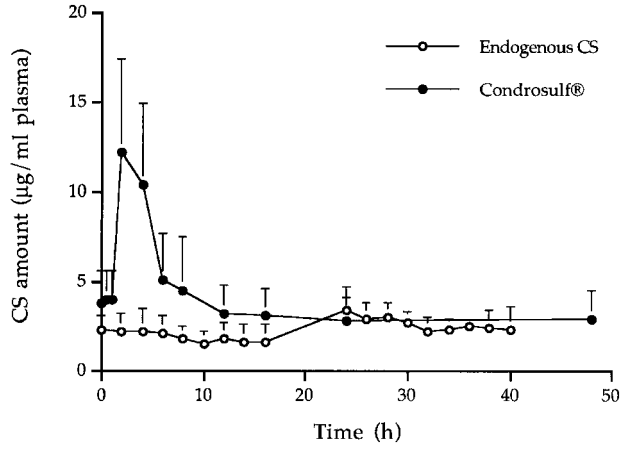

Fig. 3. Mean (*N*=6) plasma concentration of endogenous chondroitin sulfate (μg/ml plasma) and mean (*N*=20) plasma concentration of chondroitin sulfate (μg/ml plasma) determined by validated agarose-gel electrophoresis and densitometric scanning at different times after administration of 4 g bovine Condrosulf®.

Table III
Mean CS plasma pharmacokinetic parameters after administration of 4 g Condrosulf®

	C_{max} (μg/ml)	t_{max} (h)	AUC_{0-48} (μg×h/ml)	AUC_{0-24} (μg×h/ml)
Mean	12.7	2.4	179.1	111.4
S.D.	4.7	1.4	47.9	28.2
CV%	36.8	58.0	26.8	25.3
Min	5.2	2.0	75.6	66.0
Max	23.0	8.0	275.1	164.7
N	20	20	20	20

DISACCHARIDE COMPOSITION OF PLASMA CS BEFORE AND AFTER ADMINISTRATION OF CONDROSULF®

According to previous studies[29,38] the disaccharide composition of plasma CS (endogenous) at pre-dose before administration of Condrosulf® was 60.40±8.94% of non-sulfated disaccharide and 39.60±8.94% of 4-sulfated disaccharide (with a sulfate to disaccharide ratio of about 0.40) (Fig. 4).

CS of Condrosulf® has a high percentage of monosulfated disaccharides in position 4 (55.0±5.0%) and 6 (40±5.0%) of galactosamine (and about 5.0±2.0% of non-sulfated disaccharide) with a sulfate to disaccharide ratio of about 0.95[2,28,33]. The molecular mass of this polysaccharide was about 25,000–30,000 determined by HPLC[39]. After oral administration of Condrosulf® a significative decrease in the relative amount of non-sulfated disaccharide was measured (Fig. 4), reaching the minimum relative percentage of 22.96±11.68% at 4 h (Fig. 5). At the same time 4-sulfated disaccharide increased to a maximum of 60.50±10.45% at 4 h, and 6-sulfated disaccharide appeared in blood reaching a maximum concentration of 17.33±6.52% at 2 h (Fig. 5). Concomitantly the mean

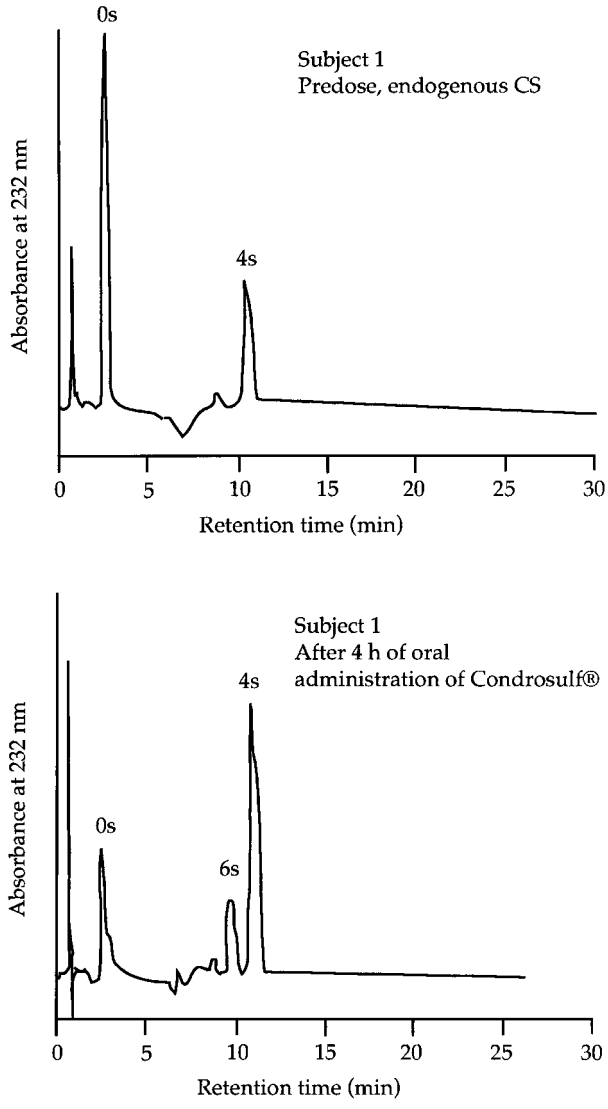

Fig. 4. SAX-HPLC chromatogram of disaccharides of endogenous chondroitin sulfate (predose) and after 4 h of oral administration of Condrosulf®. ΔDi-0s: non-sulfated disaccharide; ΔDi-6s: 6-sulfated disaccharide; ΔDi-4s: 4-sulfated disaccharide.

charge density raised from 0.40±0.09 at pre-dose to a maximum of 0.78±0.11 measured 4 h after Condrosulf® administration (Fig. 6); 48 h after drug administration the disaccharides composition almost returned to basal levels; the ΔDi-0s was 58.28±4.70%, ΔDi-4s 41.73±4.70%, charge density 0.42±0.05.

Discussion

The aim of this study was to assess the bioavailability of CS and its constituents after oral administration of Condrosulf® to 20 healthy male volunteers. The possibility of a physiological regulation of the plasma levels of endogenous CS during the day was also assessed.

Before beginning the clinical trial with Condrosulf®, we examined the type and structure of glycosaminoglycans in the plasma of healthy volunteers by the same extraction and purification approach used to purify CS after oral administration. Using unspecific proteolytic digestion with papain[29], we recovered a unique kind of glycosaminoglycan, in particular a low-sulfated chondroitin, in all volunteers. In fact, a large amount is constituted by non-sulfated chondroitin and about 40% is formed by chondroitin 4-sulfate. These results agree with other studies[40]. The structure of endogenous CS purified from plasma did not differ with male and female gender nor with age. Furthermore, results of other investigators show that human plasma or serum glycosaminoglycans do not vary with age or sex except in children, who had significantly higher values than adults[41]. As a consequence, male volunteers aged from 18 to 30 years were enrolled in the study.

The absorption of sulfated glycosaminoglycans (heparin, heparan sulfate, CS, dermatan sulfate) administered orally remains a controversial issue due to the difficulty in accepting that molecules with high molecular mass and charge density can pass the gastric and intestinal mucosa. However, various experimental findings on intestinal absorption of glycosaminoglycans are reported in literature. The experimental approach to study the metabolic fate of glycosaminoglycans after oral administration may be divided into three groups. The first group utilizes fluoroscinated[42], tritiated[27,43,44] or iodinized[45] glycosaminoglycans. The second group measures the concentration of unlabeled glycosaminoglycans (or its metabolites) by chemical methods[46], and a third group determines the biological activity of the absorbed materials[47,48]. The first and the third experimental approaches are more sensitive than the chemical techniques. Our study reports for the first time a new methodological approach to the study of the amount and physico-chemical properties and structure of CS (Condrosulf®) after oral administration. This approach has already been utilized to investigate the adsorption and modifications of the structure of dermatan sulfate after oral administration to human healthy volunteers[30]. The combination of agarose-gel electrophoresis with the determination of unsaturated disaccharides of CS by HPLC permits us to obtain quantitative and qualitative informations (the pattern of variously sulfated disaccharides and charge density) on the modifications of plasma CS after oral administration of exogenous CS (Condrosulf®). Moreover, agarose-gel electrophoresis detects polysaccharides with a molecular mass greater than about 2000 (about 3–4 disaccharide units) as a consequence of the electrostatic interactions between glycosaminoglycans and toluidine blue[49].

Endogenous CS plasma concentration remained quite constant during the 40 h sampling period, showing no daily quantitative modification. This can also be due to the controlled lifestyle of volunteers during the trial. In fact, diurnal variations in human plasma glycosaminoglycans have been reported after physical exercise[50].

It is very difficult to compare the amounts of endogenous CS measured in this study with those reported in the literature. In fact, the amounts of total or single glycosaminoglycan are generally evaluated as nmoles of hexuronic acid and not as weight. Quantitative analysis were performed by agarose-gel electrophoretic separation and densitometric analysis using calibration curves. However, the amount of CS may be underestimated due to the different primary structures of plasma CS (rich in non-sulfated disaccharides) and CS (from bovine) used to construct the calibration curves. In fact, these two polysaccharides have different charge density, and this can influence the capacity of toluidine blue (a cationic dye) to bind to polysaccharides. Nevertheless, glycosaminoglycans values ranging from about 2 to 8 μg/ml plasma/serum can

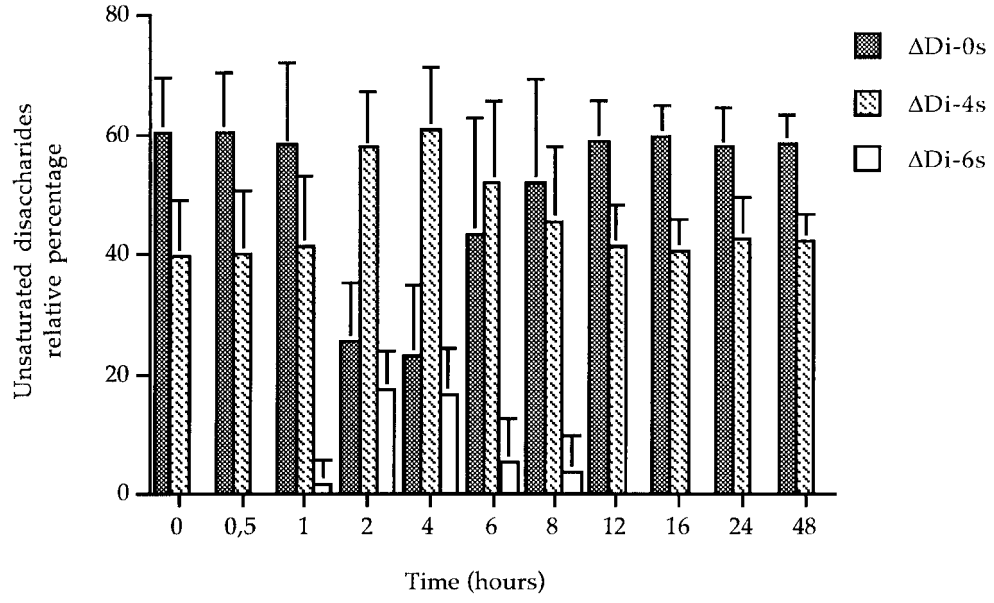

Fig. 5. Unsaturated disaccharides relative percentage of chondroitin sulfate determined by HPLC in 20 healthy human volunteers at different times after administration of 4 g bovine Condrosulf®. ΔDi-0s: non-sulfated disaccharide; ΔDi-6s: 6-sulfated disaccharide; ΔDi-4s: 4-sulfated disaccharide.

be assumed from various published data[50–52]. In this study, basal values or predose values of endogenous CS were calculated to be from 1.4 to 7.6 μg/ml plasma (from 1.4 to 7.6 μg/ml plasma for predose values and from 3.2 to 5.3 μg/ml plasma for basal values).

The results presented indicate that exogenous CS becomes available in the bloodstream after oral administration of Condrosulf®. This is proved by the up to 263% increase over baseline levels reached after administration of bovine extracted Condrosulf®. The peak increase was statistically significant with respect to the levels measured at baseline or immediately after baseline. Endogenous CS plasma concentration remained quite constant during the 40 h sampling period thus confirming that the increase measured after drug administration was due to CS absorption and not to physiological regulation of CS levels. Moreover the absorption of exogenous CS was proved by the change in the composition of unsaturated disaccharides found in plasma after drug administration with respect to baseline with a decrease of non-sulfated disaccharide and a concomitant increase of 4-sulfated disaccharide and appearance of 6-sulfated disaccharide. Concomitantly, the mean sulfate to disaccharide ratio increased from 0.40±0.09 to 0.78±0.11 after Condrosulf® administration. Furthermore, CS reaches the blood compartment with a molecular mass greater than about 2000 as determined by agarose-gel electrophoresis. On the other hand, we already demonstrated that a large number of dermatan sulfate species with molecular mass from about 7500 to 20,000 are present in normal human plasma after oral absorption of this polysaccharide[30]. After oral absorption of Condrosulf® a large number of CS species with molecular mass of about 5000–15,000 is detected in blood. Moreover, very low-molecular mass species are detected, with a prevalence of oligosaccharides (data not shown, study in progress).

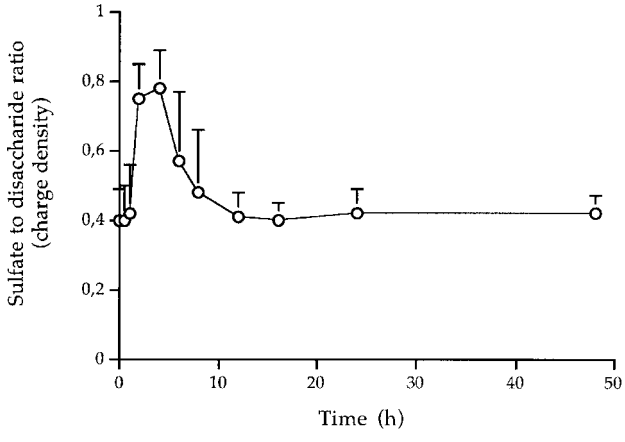

Fig. 6. Charge density (sulfate to disaccharide ratio) of chondroitin sulfate determined in 20 healthy human volunteers at different times after administration of 4 g bovine Condrosulf®.

Previous studies have demonstrated that after oral absorption the mean plasma curve of exogenous CS peaks after 3.2 h[27], 4.0 h[53], or 5.0 h[28]. In this trial, Condrosulf® is quickly absorbed with a t_{max} of 2.4 h. On the other hand, the increase in CS plasma level is significant from 2 to 6 h after drug administration.

This research extends previous results obtained by other researchers in man and experimental animals, both with CS and other polysaccharides[27,28,30,42–45,47,53,54] confirming that molecules possessing high molecular mass and charge density can be absorbed orally. On the other hand, quantitative evaluations, such as C_{max}, t_{max}, AUC, and bioavailability, can depend on the nature of the drug absorbed by the oral route (molecular mass, charge density, cluster of disulfated disaccharides), which can affect quantitative assays. Moreover, after oral absorption, glycosaminoglycans can bind to vascular components and not be immediately available for quantitative assays (as demonstrated by Jaques *et al*.[55], who reported that only a small fraction of orally administrated heparin and dextran sulfate that enter the body is recovered from plasma and that the endothelium rapidly removes these drugs from blood).

Furthermore, the presence in the human intestinal microflora of the *bacteroides stercoris* able to degrade the CS has been documented[56].

From this study and literature data, it appears that exogenous CS (Condrosulf®) is absorbed as high molecular mass polysaccharide together with derivatives resulting from a partial depolymerization and desulfation. These results are further confirmed by the therapeutic effects of Condrosulf®[14–26] and other sulfated polysaccharides[47,57,58] when administered orally.

References

1. Imanari T, Toida T, Koshiishi I, Toyoda H. High-performance liquid chromatographic analysis of glycosaminoglycan-derived oligosaccharides. J Chromatogr A 1996;720:275–93.
2. Mucci A, Schenetti L., Volpi N. ^{1}H and ^{13}C nuclear magnetic resonance identification and characterization of components of chondroitin sulfates of various origin. Carbohydr Polymers 2000;41:37–45.
3. Cassaro CMF, Dietrich CP. Distribution of sulfated mucopolysaccharides in invertebrates. J Biol Chem 1977;252:2254–61.
4. Ruoslathi E. Structure and biology of proteoglycans. Ann Rev Cell Biol 1988;4:229–55.
5. Kjellen L, Lindahl U. Proteoglycans: structures and interactions. Annu Rev Biochem 1991;60:443–75.
6. Sugahara K, Tanaka Y, Yamada S, Seno N, Kitagawa H, Haslam SM, *et al*. Novel sulfated oligosaccharides containing 3-O-sulfated glucuronic acid from king krab cartilage chondroitin sulfate K. Unexpected degradation by chondroitinase ABC. J Biol Chem 1996;271:26745–54.
7. Brittis PA, Canning DR, Silver J. Chondroitin sulfate as a regulator of neuronal patterning in the retina. Science 1992;255:733–6.
8. Barkalow FJ, Schwarzbauer JE. Interactions between fibronectin and chondroitin sulfate are modulated by molecular context. J Biol Chem 1994;269: 3957–62.
9. Nadanaka S, Clement A, Masayama K, Faissner A, Sugahara K. Characteristic hexasaccharide sequences in octasaccharides derived from shark cartilage chondroitin sulfate D with a neurite outgrowth promoting activity. J Biol Chem 1998;273:3296–307.
10. Iida J, Meijne ML, Oegema TR Jr, Yednock TA, Kovach NL, Furcht LT, *et al*. A role of chondroitin sulfate glycosaminoglycan binding site in $\alpha_4\beta_1$ integrin-mediated melanoma cell adhesion. J Biol Chem 1998;273:5955–62.
11. Rachmilewitz J, Tykocinski ML. Differential effects of chondroitin sulfates A and B on monocyte and B-cell activation: evidence for B-cell activation via a CD44-dependent pathway. Blood 1998;92:223–9.
12. Sakai T, Kyogashima M, Kariya Y, Urano T, Takada Y, Takada A. Importance of GlcUAβ1-3GalNAc(4s,6s) in chondroitin sulfate E for t-PA- and u-PA-mediated Glu-plasminogen activation. Thromb Res 2000;100: 557–65.
13. Kuettner KE, Schleyerbach R, Peyron JG, Hascall VC. Articular Cartilage and Osteoarthritis. New York: Raven Press 1991.
14. Morreale P, Manopulo R, Galati M, Boccanera L, Saponati G, Bocchi L. Comparison of the antiinflammatory efficacy of chondroitin sulfate and diclofenac sodium in patients with knee osteoarthritis. J Rheumatol 1996;23:1385–91.
15. Ronca F, Palmieri L, Panicucci P, Ronca G. Anti-inflammatory activity of chondroitin sulfate. Osteoarthritis Cart 1998;6(Suppl A): 14–21.
16. Pipitone VR. Chondroprotection with chondroitin sulfate. Drugs Exptl Clin Res 1991;XVII(1): 3–7.
17. Paroli E. Glycosaminoglycan chondroprotection: pharmacological vistas. Int J Clin Pharm Res 1993;XIII:1–9.
18. McNamara PS, Barr SC, Erb HN. Hematologic, hemostatic, and biochemical effects in dogs receiving an oral chondroprotective agent for thirty days. Am J Vet Res 1996;57:1390–4.
19. Uebelhart D, Thonar E, Zhang J, Williams JM. Protective effect of exogenous chondroitin 4,6-sulfate in the acute degradation of articular cartilage in the rabbit. Osteoarthritis Cart 1998;6(Suppl A): 6–13.
20. Rovetta G. Galactosaminoglycuronoglycan sulfate (matrix) in therapy of tibiofibular osteoarthritis of the knee. Drugs Exptl Clin Res 1991;XVII:53–7.
21. Fioravanti A, Franci A, Anselmi F, Fattorini L, Marcolongo R. Clinical efficacy and tolerance of galactosaminoglucuronoglycan sulfate in the treatment of osteoarthritis. Drugs Exptl Clin Res 1991;XVII:41–4.
22. Coaccioli S, Allegra A, Pennacchi M, Mattioli C, Ponteggia F, Brunelli A, *et al*. Galactosaminoglucuronoglycan sulphate in the treatment of osteoarthritis: clinical efficacy and tolerance of oral and intra-articular administration. Int J Clin Pharm Res 1998;XVIII:39–50.
23. Verbruggen G, Goemaere S, Veys EM. Chondroitin sulfate: S/DMOAD (structure/disease modifying anti-osteoarthritis drug) in the treatment of finger joint OA. Osteoarthritis Cart 1998;6(Suppl A): 37–8.
24. Uebelhart D, Thonar E, Delmas PD, Chantraine A, Vignon E. Effects of oral chondroitin sulfate on the progression of knee osteoarthritis: a pilot study. Osteoarthritis Cart 1998;6(Suppl A): 39–46.
25. Bourgeois P, Chales G, Dehais J, Delcambre B, Kunz J-L, Rozenberg S. Efficacy and tolerability of chondroitin sulfate 1200 mg/day vs chondroitin sulfate 3×400 mg/day vs placebo. Osteoarthritis Cart 1998;6(Suppl A): 25–30.
26. Bucsi L, Poor G. Efficacy and tolerability of oral chondroitin sulfate as a symptomatic slow-acting drug for osteoarthritis (SYSADOA) in the treatment of knee osteoarthritis. Osteoarthritis Cart 1998;6(Suppl A): 31–6.
27. Conte A, De Bernardi M, Palmieri L, Lualdi P, Mautone G, Ronca G. Metabolic fate of exogenous chondroitin sulfate in man. Arzneim-Forsch/Drug Res 1991;41:768–72.
28. Conte A, Volpi N, Palmieri L, Bahous I, Ronca G. Biochemical and pharmacokinetic aspects of oral treatment with chondroitin sulfate. Arzneim-Forsch/ Drug Res 1995;45:918–25.
29. Volpi N, Cusmano M, Venturelli T. Qualitative and quantitative studies of heparin and chondroitin sulfates in normal human plasma. Biochim Biophys Acta 1995;1243:49–58.
30. Volpi N. Physico-chemical properties and the structure of dermatan sulfate fractions purified from plasma after oral administration in healthy human volunteers. Thromb Haem 1996;75(3): 491–6.

31. Volpi N. 'Fast moving' and 'slow moving' heparins, dermatan sulfate and chondroitin sulfate: qualitative and quantitative analysis by agarose-gel electrophoresis. Carb Res 1993;247:263–78.
32. Volpi N. Fractionation of heparin, dermatan sulfate, and chondroitin sulfate by sequential precipitation: a method to purify a single glycosaminoglycan species from a mixture. Analyt Biochem 1994;218: 382–91.
33. Volpi N. Hyaluronic acid and chondroitin sulfate unsaturated disaccharides analysis by high-performance liquid chromatography and fluorimetric detection with dansylhydrazine. Anal Biochem 2000;277:19–24.
34. Volpi N, Sandri I, Venturelli T. Activity of chondroitin ABC lyase and hyaluronidase on free-radical degraded chondroitin sulfate. Carb Res 1995;279: 193–200.
35. Wagner JG. Fundamentals of Clinical Pharmacokinetics. Hamilton, IL: Drug Intelligence Publications 1975.
36. Gibaldi M. Biopharmaceutics and Clinical Pharmacokinetics. Philadelphia: Lea and Febiger 1984.
37. Rowland M, Tozer T. Clinical Pharmacokinetics. Concepts and Applications. 2nd ed. Philadelphia: Lea and Febiger 1988.
38. Murata K, Horiuchi Y. Molecular weight-dependent distribution of acidic glycosaminoglycans in human plasma. Clin Chim Acta 1977;75:59–69.
39. Volpi N, Bolognani L. Glycosaminoglycans and proteins: different behaviour in size exclusion-high performance liquid chromatography. J Chromatogr A 1993;630:390–6.
40. Staprans I, Felts JM. Isolation and characterization of glycosaminoglycans in human plasma. J Clin Invest 1985;76:1984–91.
41. Calatroni A, Donnelly PV, Di Ferrante N. The glycosaminoglycans of human plasma. J Clin Inv 1969;48:332–43.
42. Pescador R, Diamantini G, Mantovani M, Malandrino S, Riva A, Casu B, Oreste P. Absorption by the rat intestinal tract of fluorescein-labelled pig duodenal glycosaminoglycans. Arzneim-Forsch 1980;30: 1893–6.
43. Larsen AK, Lund DP, Langer R, Folkman J. Oral heparin results in the appearance of heparin fragments in the plasma of rats. Proc Natl Acad Sci USA 1986;83:2964–8.
44. Palmieri L, Conte A, Giovannini L, Lualdi P, Ronca G. Metabolic fate of exogenous chondroitin sulfate in the experimental animal. Arzneim-Forsch 1990;40: 319–23.
45. Dawes J, Hodson BA, Pepper DS. The absorption, clearance and metabolic fate of dermatan sulphate administered to man—studies using a radioiodinated derivative. Thromb Haemost 1989;62:945–9.
46. Baici A, Horler D, Moser B, Hofer HO, Fehr K, Wagenhauser FJ. Analysis of glycosaminoglycans in human serum after oral administration of chondroitin sulfate. Rheumatol Int 1992;12:81–8.
47. Crepaldi G, Rossi A, Coscetti G, Abbruzzese E, Calveri U, Calabró A. Sulodexide oral administration influences blood viscosity and fibrinolysis. Drugs Exptl Clin Res 1992;18:189–95.
48. Scagnol I, Fumagalli G, Andriuoli G. Anticoagulant and antithrombotic activity of heparin salts by intraduodenal route in rabbits. Thromb Res 1992;68:195–200.
49. Volpi N. Characterization of different relative molecular mass heparins (from 11,600 to 1,600) by various analytical techniques. J Cromatogr Biomed Appl 1993;622:13–20.
50. Muraca U, Vinci R, Ferlazzo AM, Muraca G, Calatroni A. Factors affecting glycosaminoglycan concentration in normal human plasma. Ital J Biochem 1992;41:159–69.
51. Dietrich CP, Martins JRM, Sampaio LO, Nader HB. Anomalous structure of urinary chondroitin sulfate from cancer patients. Lab Invest 1993;4:439–45.
52. Niebes P, Schifflers MH. Micromethod for the determination of glycosaminoglycans in the serum. Results from the serum of healthy and varicose subjects. Clin Chim Acta 1975;62:195–202.
53. Ronca G, Conte A. Metabolic fate of partially depolymerized shark chondroitin sulfate in man. Int J Clin Pharm Res 1993;XIII(Suppl): 27–34.
54. Dawes J, McLaren M, Forbes C, Belch JJF, Lane DA, Bray B, *et al*. The pharmacokinetics of dermatan sulphate MF701 in healthy human volunteers. Br J Clin Pharmac 1991;32:361–6.
55. Jaques LB, Hiebert LM, Wice SM. Evidence from endothelium of gastric absorption of heparin and of dextran sulfates 8000. J Lab Clin 1991;117:122–30.
56. Ahn MY, Shin KH, Kim D-H, Jung E-A, Toida T, Linhardt RJ, Kim YS. Characterization of a bacteroides species from human intestine that degrades glycosaminoglycans. Can J Microbiol 1998;44: 423–9.
57. Vasdev S, Sampson CA, Longerich L, Parai S. Oral heparin prevents hypertension and elevated cytosolic calcium in salt-sensitive rats. Artery 1992;19: 225–45.
58. Baggio B, Gambaro G, Marzaro G, Marchini F, Borsatti A, Crepaldi G. Effects of the oral administration of glycosaminoglycans on cellular abnormalities associated with idiopathic calcium oxalate nephrolithiasis. Eur J Clin Pharm 1991;40:237–40.

Appendix

The objective was to establish and validate an agarose-gel electrophoresis assay for the determination of Condrosulf® in human plasma. The validation was performed in compliance with Good Laboratory Practice Standards. Agarose-gel electrophoresis assay has been developed by the author and several scientific papers have been published on this topic[31,32,49].

AGAROSE-GEL ELECTROPHORESIS CALIBRATION SAMPLES. CALIBRATION CURVE

For the agarose-gel electrophoresis assay validation, four calibration samples of Condrosulf® (from IBSA, lot no. 93150) with the following concentration levels were prepared: 100 μg/ml (1 μg/10 μl), 300 μg/ml (3 μg/10 μl), 500 μg/ml (5 μg/10 μl) and 700 μg/ml (7 μg/10 μl). The solutions were divided in 0.500 ml aliquots and stored in 'Eppendorf' polypropylene vials below −18°C until analysis. Different stock and working solutions were used for samples preparation. These solutions were prepared by a qualified person not involved in the preparation of the stock solution for the calibration samples. Calibration curve from 1 to 7 μg of CS (Condrosulf®) was performed. Calibration

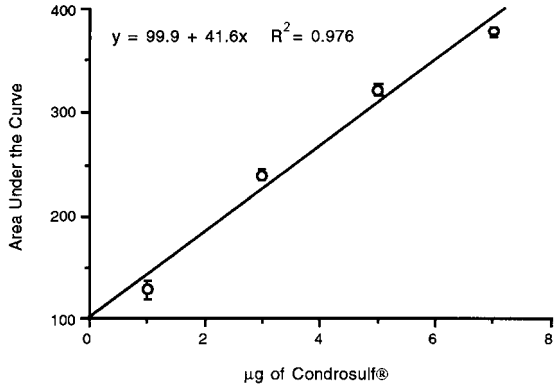

Fig. A1. The calibration curve with the equation parameters and the coefficient of correlation.

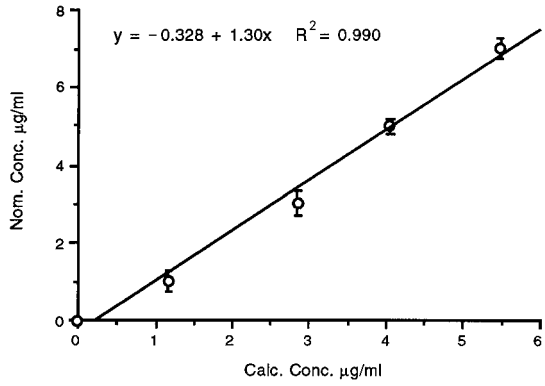

Fig. A2. The back calculated calibration curve of Condrosulf® in human plasma with the equation parameters and the coefficient of correlation.

curves were calculated by using the Regression software for Macintosh computer, version M 1.23, and the StatWorks software for Macintosh computer, version 1.2.

Figure A1 shows the calibration curve with the equation parameters and the coefficient of correlation. The within-batch precision (coefficient of variation) of quality control samples was in the range from 3.7% to 16.5% (number of replicates=5). The limit of quantification was set at 1 µg for Condrosulf®. Five replicates of the lowest standard were analyzed to evaluate the limit of quantification. The calibration curves were linear with a coefficient of correlation higher than 0.960 (mean 0.976).

AGAROSE-GEL ELECTROPHORESIS CALIBRATION SAMPLES OF CONDROSULF® IN HUMAN PLASMA. CALIBRATION CURVE

For the agarose-gel electrophoresis assay validation of Condrosulf® in human plasma, four calibration samples of Condrosulf® with the following concentration levels were prepared: 100 µg/ml (1 µg/10 µl), 300 µg/ml (3 µg/10 µl), 500 µg/ml (5 µg/10 µl) and 700 µg/10 ml (7 µg/µl) and mixed to 1.0 ml of normal human plasma. The solutions were stored in 'Eppendorf' polypropylene vials below −18°C until analysis.

Condrosulf® at different concentration (from 0 to 7 µg) was extracted and purified from 1.0 ml human plasma according to the method reported (see Experimental section). After extraction and purification, the amounts of Condrosulf® in human plasma were calculated by agarose-gel electrophoresis against the calibration curve constructed with increasing amounts (from 1 to 7 µg) of Condrosulf®.

Figure A2 shows the back calculated calibration curve with the equation parameters and the coefficient of correlation. The within-batch precision (coefficient of variation) of quality control samples was in the range from 5.4% to 22.1% (predose) (number of replicates=6). The within-batch accuracy of quality control samples without the basal value (predose) of endogenous CS was in the range from −22% to +18% (number of replicates=6). The within-batch precision (coefficient of variation) of quality control samples without the basal value (predose) of CS was in the range from 10.6% to 55.6% (number of replicates=6).

CS from Condrosulf® was identified by its specific coefficient of migration in agarose-gel electrophoresis. Six different human blank (predose) plasma samples were tested for the presence of endogenous CS. All plasma samples showed the presence of endogenous CS in the range of 1.7 to 3.3 µg/ml.

RECOVERY [%] OF CHONDROSULF® FROM NORMAL HUMAN PLASMA

The extraction recovery was determined by comparing the areas of pure Condrosulf® solutions with those from extracted samples at two different concentrations. The recovery for Condrosulf® was found to be 95.0% at 3 µg and 81.0% at 5 µg.

Abbreviations

CS, chondroitin sulfate.
ΔDi-0s: 2-acetamido-2-deoxy-3-0-(4-deoxy-α-L-*threo*-hex-4-enepyranosyluronic acid)-D-galactose.
ΔDi-4s: 2-acetamido-2-deoxy-3-0-(4-deoxy-α-L-*threo*-hex-4-enepyranosyluronic acid)-D-galactose 4-sulfate.
ΔDi-6s: 2-acetamido-2-deoxy-3-0-(4 -deoxy-α-L-*threo*-hex-4-enepyranosyluronic acid)-D-galactose 6-sulfate.