HPLC Determination of Chondrosine in Mouse Blood Plasma after Intravenous or Oral Dose

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Received January 31, 2007; accepted April 12, 2007

The bioavailability of chondrosine was evaluated by its direct measurement as found in the blood plasma following removal of plasma proteins by perchloric acid. The postcolumn HPLC determination of chondrosine was performed on an SCX column (6 mm i.d.×150 mm), 0.35 mol/l boric acid (pH 5.2 adjusted by 0.1 mol/l NaOH) as an eluent (0.9 ml/min), 0.5% 2-cyanoacetamide and 1.0 mol/l NaOH as fluorogenic reagents (0.25 ml/min each) with a fluorescence detector (ex. 331 nm, em. 383 nm). Two separate animal studies were conducted. In study 1, adult male ddY mice (n=6) received i.v. chondrosine (1.0 mg/kg body weight) and the plasma samples were collected. In the second study, 6 adult male ddY mice received p.o. chondrosine (400 mg/kg body weight) and the plasma samples were collected. Blood plasma samples were deproteinized by perchloric acid, analyzed and the bioavailability of chondrosine was determined. Twenty five to fifty microliters of blood plasma were required for the assay. Chondrosine was absorbed after oral administration with two phases having two maximum values, 7.8±0.4 and 4.0±1.9 at 15 μg/ml and 120 min, respectively; it disappeared from the blood flow very quickly after intravenous administration. This study provides the first report of the bioavailability of orally administered chondrosine in mice.

Key words  chondrosine; HPLC determination; oral dose; bioavailability

Osteoarthritis is a heterogeneous condition with various clinical expressions.1,2) The most common symptoms are pain and functional disability resulting from destructive changes of the osteoarthritic joint.1—3) Current treatment of osteoarthritis is not aimed at a cure but at palliative management including physical, pharmacological, and surgical approaches.4) Medicinal treatment includes analgesics and symptomatic slow-acting drugs.5) The latter class of compounds has a slow onset of action and improve osteoarthritis symptoms.6) Some of them are administered orally and some are used intra-articularly. Among these materials, chondroitin sulfate (CS) has proven to be a valuable therapeutic tool for the symptomatic treatment of osteoarthritis.7,8) Several controlled trials have shown its effects as a nutraceutical with application in the therapy of osteoarthritis of the knee and in articular cartilage osteoarthritis with high tolerability.9—10) Although we have published a new mechanism for the anti-inflammatory and chondroprotective properties of CS that may be useful in designing new therapeutic applications for its use in the treatment of immediate-type hypersensitivity,11—13) it is also very difficult to strictly regulate its molecular weight.14)

On the other hand, chondrosine [β-D-glucopyranuronic acid-(1→3)-2-amino-2-deoxy-D-galactopyranose] is derived from the repeating unit of CS. Chondrosine can be readily obtained from the parent polysaccharide by acid catalyzed hydrolysis,15 a process that also results in desulfation and N-deacetylation of the disaccharides. Furthermore, previous study has shown that chondrosine increased the uptake of 35S labeled sulfate at rat cartilage, while CS showed a negative effect of 35S level at that tissue compared to that of control group.16) Based on this observation, the absorption and blood plasma concentration of orally and intravenously administered chondrosine was planned. However, one of the major challenges associated with assessing the bioavailability of chondrosine has been the lack of sensitive analytical methods that can quantify this compound in biological matrices.

In this paper, we have newly established a sensitive and specific determination postcolumn HPLC method for chondrosine using 2-cyanoacetamide as a fluorogenic reagent,17,18) and the bioavailability of chondrosine in mice was investigated.

MATERIALS AND METHODS

Materials  Chondrosine was prepared according to the method described previously.16,17) A chondroitin sulfate sample (average molecular weight (MWavg) 15000 from bovine tracheal cartilage) for preparation of chondrosine was purchased from Shin-Nippon Yakugyo Co. (Japan). Chondroitin sulfate (MWavg, 31000) from shark cartilage was kind gift from Seikagaku (Tokyo). Centrifugal filters (BIOMAX-5) having a molecular weight cut-off of 5000 were purchased from Millipore Japan (Tokyo). All other chemicals were of analytical grade.

HPLC Analysis  The postcolumn HPLC system for determination of chondrosine was constructed with a PU-980 intelligent HPLC pump (Jasco, Tokyo, Japan), a double-plunger pump for the fluorogenic reagent solution (SPU-2.5W; Shimamura Instruments, Tokyo), a sample injector with a 20 μl loop (Model 7725i; Reodyne, CA, U.S.A.), a fluorescence spectrophotometer (FP-1520S; Jasco), a column switching system (Mini-80; Taittech, Tokyo), a chromatointe-
Chondrosine dissolved in saline solution (1.0 mg/ml) was injected through the tail vein at a dose of 5.0 mg/kg body weight (ca. 100 μg each animal). Blood samples were collected from the eye socket vein at 0 to 240 min after the administration. Blood samples (about 30 μl) were collected in 50 μg heparin at the following times: 0 (before injection), 5, 10, 20, 30, 45, 60, 120, and 240 min after intravenous administration. Each collected sample was immediately centrifuged at 1500 g for 10 min to obtain plasma, which was transferred to a polypropylene plastic vial and stored in a deep freezer until used.

On the other hand, chondrosine was also orally administered at a dose of 400 mg/kg body weight. Blood samples were collected from the eye socket vein at 0 to 480 min after the administration. Plasma samples were pretreated as described above.

RESULTS AND DISCUSSION

Determination of Chondrosine by the Post Column HPLC Different concentrations and pH conditions of boric acid solution were tested for the rapid separation of chondrosine from interferences, and the HPLC conditions were finally optimized as shown in Materials and Methods.

Pretreatment for Determination of Chondrosine in Blood Plasma The chromatograms of the blank mouse plasma and a plasma sample spiked with chondrosine are shown in Fig. 1. No interferences were detected at the retention times of chondrosine.

The relationship between the peak heights and appropriate concentrations was linear over the tested range (1—150 μg/ml). The correlation coefficient (r²) of all calibration curves was over 0.996. A representative chromatogram of the sample spiked with chondrosine is shown in Fig. 1.

The recovery at the two concentrations of chondrosine samples (40, 200 μg/ml) are shown in Table 1. The standard deviations of reproducibility on intra- and interday analyses are also satisfactory (below 5%). The lower limit of quantification was determined to be 0.2 μg/ml. The results indicated a satisfying recovery of chondrosine from plasma samples of the assay according to the given pretreatment procedure.

In a pilot experiment, the plasma concentration–time profile of chondrosine after intravenous administration was determined. After injection of the chondrosine at a dose of 5.0 mg/kg body weight, the following concentration–time profile was obtained (Fig. 2). Relatively fast distribution and the recovery of chondrosine was calculated to obtain intra- and interday variation. The lower limit of quantification was estimated to be 0.2 μg/ml in the original plasma sample, and the analyte response at this level should be at least five times higher than that of the noise level.
Since there are low sulfated chondroitin sulfate chains, which consist of 35% unsulfated and ca. 65% 4-O-sulfated disaccharide units, and no 6-O-sulfated disaccharide unit, bound to inter-alpha-trypsin inhibitor in blood plasma,23) we investigated the absorption of orally administered shark chondroitin sulfate to detect 6-O-sulfated disaccharide units as a marker. As we expected, few 6-O-sulfated disaccharide units were detected. For example, less than 0.02 µg/ml of shark chondroitin sulfate was detected from only 1 of 5 mice blood plasma, and no 6-O-sulfated disaccharide unit was found from another plasma samples obtained at 8 h after oral administration of chondroitin sulfate (data not shown).

Carefully organized animal experiments are required to confirm the bioavailability of orally administered chondroitin sulfate, however, chondrosine may be suitable as a nutraceutical/supplement in comparison with chondroitin sulfate.

CONCLUSION

In this study, for the first time a simple, precise and accurate HPLC method suitable for the determination of chondrosine in plasma was established. Prior to HPLC analysis, a deproteinization procedure under acidic conditions was performed to effectively isolate chondrosine from mouse plasma. The method was validated with respect to selectivity, linearity, and recovery, and its applicability was confirmed by analysis of mouse plasma sample obtained from oral and intravenous administrations of chondrosine to mice. The method allowed us to depict a concentration–time profile after i.v. and p.o. administrations. This study provides the first information about the plasma concentration–time profile of chondrosine in mice.

Acknowledgement We are grateful to Professor Robert J. Linhardt at Rensselaer Polytechnic Institute (U.S.A.) for the improvement of English.

REFERENCES


Table 1. Recovery of Spiked Chondrosine from Mouse Plasma

<table>
<thead>
<tr>
<th>Sample</th>
<th>Spiked chondrosine (µg/ml)</th>
<th>Found (µg/ml)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>0.0</td>
<td>0</td>
<td>___</td>
</tr>
<tr>
<td>Group 2</td>
<td>40.0</td>
<td>40.6±0.5</td>
<td>101.5</td>
</tr>
<tr>
<td>Group 3</td>
<td>200.0</td>
<td>203.0±3.6</td>
<td>101.5</td>
</tr>
</tbody>
</table>

Each value obtained from triplicate experiments.

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Fig. 2. Time Course of Chondrosine in Mouse Blood Plasma after Intravenous Administration

Fig. 3. Time Course of Chondrosine in Mouse Blood Plasma after Oral Administration


