Evidence of the Oral Absorption of Chondroitin Sulfate as Determined by Total Disaccharide Content After Oral and Intravenous Administration to Horses

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Disaccharides formed specifically from the breakdown of chondroitin sulfate were found in horse plasma samples after the oral dosing of an 8.0 and 16.9 kDa molecular weight chondroitin sulfate. These results suggest that chondroitin sulfate, or fragments of this molecule, are absorbed after oral administration. Authors’ addresses: Pharmacokinetics-Biopharmaceutics Laboratory, Department of Pharmaceutical Sciences, University of Maryland School of Pharmacy, 100 Penn Street, AHB 540A, Baltimore, MD 21201 (Eddington and Du) and Marion duPont Scott Equine Medical Center, Leesburg, VA 20177 (White). © 2001 AAEP.

1. Introduction

The oral absorption of the glycosaminoglycan (GAG), chondroitin sulfate (CS), has been consistently debated over the years. There are limited studies which report the bioavailability of intact CS, and no studies to date have demonstrated absorption in the horse. This may be because of the lack of sensitive analytical methods, or that chondroitin sulfate may not be absorbed as an intact molecule. CS is a large molecule and its bioavailability has been questioned; however, when given orally, there is evidence of decreasing pain and slowing of the progression of osteoarthritis in herbivores. Nonetheless, its molecular weight would suggest that absorption across the gastrointestinal mucosa, which contains a variety of GAG-degrading enzymes such as exoglycosidases, sulfatases, and hyaluronidase-like enzymes, is low. Conceivably, the CS molecule may be metabolized in the liver to unsaturated disaccharide units prior to reaching the systemic circulation after oral dosing. Studies have reported that CS is metabolized to 3 primary unsaturated disaccharides: (1) 2-acetamido-2-deoxy-3-O-(β-D-glucuronic acid)-D-galactose (ΔDi-OS), (2) 2-acetamido-2-deoxy-3-O-(β-D-glucuronic acid)-4-O-sulpho-D-galactose (ΔDi-4S), and (3) 2-acetamido-2-deoxy-3-O-(β-D-glucuronic acid)-6-O-sulpho-D-galactose (ΔDi-6S). One factor that may affect the absorption of CS is the chain length of the molecule. Recent in vitro studies using the Caco-2 cell culture system suggested that the molecular weight of CS has a direct influence on its permeability across the gastrointestinal tract, where higher permeability was reported for CSs with lower molecular weight. As previously stated, one of the major chal-
lenges associated with assessing the oral absorption of CS has been the lack of sensitive analytical methods that can quantify this compound in biological matrices. To overcome this problem, assays have been developed that detect disaccharides formed from CS after treatment with chondroitinase ABC. The resultant disaccharides formed after enzymatic treatment are detected using ultraviolet or fluorescent methods. Therefore, this approach has the potential of assessing the oral absorption of CS by completely converting this compound to disaccharides. Taken together it would appear that a definitive determination of CS absorption should be the detection of its breakdown products (Di-OS, Di-4S, and Di-6S), the unsaturated disaccharides in plasma after oral administration.

Hence, the purpose of this study was 2-fold: (1) to determine the oral bioavailability of CS by determining the disaccharides formed after oral administration and (2) to determine the influence of the molecular weight of CS on its oral bioavailability again as measured by the bioavailability of disaccharides.

2. Materials and Methods
Adult horses (n = 10) were used in the study and were obtained from the Marion duPont Scott Equine Medical Center. Horses were in good health as determined by physical examination and clinical laboratory tests. Horses were kept in a 90 acre pasture or smaller paddocks at the Equine Center during periods before and between dose administration. This was an open, randomized 4-way crossover study in normal horses (n = 10). Ten horses received each of the following 4 treatments on separate occasions: (1) intravenous (IV) CS (3 g of 8 kDa, 375 μM), (2) oral (PO) CS (3 g of 8 kDa), (3) IV CS (3 g of 16.9 kDa, 176 μM), and (4) PO CS (3 g of 16.9 kDa). The 8 kDa material was an experimental material and is not commercially available. The 16.9 kDa material is commercially available (TRH122™) in Cosequin® Equine Powder. At least a 7 day washout period separated all treatments. Food was withheld from the animals for 3 hours prior to baseline sampling and 3 hours after dose administration. All horses had a catheter placed in the left jugular vein prior to sampling. The IV doses were administered in the right jugular vein at time zero and samples were collected for 24 hours. The oral dose was administered via a nasogastric tube. Sampling (20 ml) scheme was as follows: 5, 10, 15, 20, 30, and 45 minutes and 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 10, 12, and 24 hours. A validated high-performance liquid chromatography (HPLC) method using pre-column derivatization and fluorometric detection (excitation wavelength of 350 nm and emission at 530 nm) was used to quantify disaccharides (Di-OS, Di-4S, and Di-6S) in plasma. All plasma samples were treated with chondroitinase ABC in 1 mM Na2HPO4 buffer at pH 7 for 3 hours and subsequently reacted with dansylhydrazine and injected onto the chromatographic system. It should be noted that chondroitinase ABC degrades CS up to the unsaturated disaccharide (ΔDi-OS, ΔDi-4S, and ΔDi-6S). The chromatographic conditions consisted of a μ-Bondapack NH2 column, mobile phase of acetonitrile: 100 mM acetate buffer, pH 5.6 (90:20) and a flow rate of 2.0 ml/min. Noncompartmental pharmacokinetic analysis was performed on the total disaccharides formed (ΔDi-OS, ΔDi-4S, and ΔDi-6S) after IV and PO dosing. The following pharmacokinetic parameters were determined for total disaccharide concentration: area under the plasma concentration time curve (AUC), maximum plasma concentration (Cmax), time of maximum plasma concentration (Tmax), elimination half-life (t1/2), and apparent bioavailability (Fa). The Fa was defined as [AUCtotal disaccharides]PO/AUCtotal disaccharides]IV * 100. The pharmacokinetic parameters were estimated using the nonlinear least-squares regression program, Winnonlin™. The pharmacokinetic parameters were compared across treatments using analysis of variance (ANOVA) with post-hoc analysis (Dunnett). Statistical significance was assessed at a level of p < 0.05.

3. Results and Discussion
Peaks of ΔDi-OS, ΔDi-4S, and ΔDi-6S in the chromatogram from plasma samples and standards were identified by comparing their retention time with those obtained with the disaccharide standards. Blank runs with horse plasma did not display any interfering peaks. The intra-day and inter-day assay precision (RSD) ranged from 1.2% to 8.1% and from 4.3% to 5.8% for intra- and inter-day accuracy, respectively. Each of the disaccharides were found in plasma after oral and intravenous administration of the 8.0 and 16.9 kDa CS. Total disaccharide plasma concentration was highest after the intravenous dosing of the 16.9 kDa CS. There are no reports on the circulating concentrations of these disaccharides in horses. Each of these disaccharides are found in man; however, higher concentrations of both the ΔDi-4S and ΔDi-OS have been reported with trace levels of ΔDi-6S. The pharmacokinetic parameters obtained after noncompartmental analysis of the PO and IV dosing data are summarized in Table 1.

The extent of absorption of CS as indicated by the Cmax and AUC of total disaccharides after dosing with both the 8.0 and 16.9 kDa provides evidence that CS of either 8.0 or 16.9 kDa are absorbed orally. The absorption of larger molecular weight CS is unknown; however, other work in this laboratory suggests that CS of a higher molecular weight would have lower absorption. Several studies have examined the oral and parenteral absorption of GAGs including CS with inconsistent results. Absorption and nonabsorption of CS1,10-12 have been claimed. As seen in Table 1, mean Cmax and AUC were statistically higher for the 16.9 kDa CS as
compared with 8.0 kDa. However, the bioavailability of the higher molecular weight CS was lower numerically, but not statistically (22%) as compared with the 8.0 kDa (32%). It should be noted that 375 µM of the 8.0 kDa CS was administered as compared with 176 µM of the 16.9 kDa. Based on this, a higher concentration of total disaccharides would be expected from the lower molecular weight CS. This was not observed; however, the bioavailability associated with the 8.0 kDa was higher than the 16.9 kDa CS, which would suggest a higher conversion of the disaccharides. Reasons for this difference may be based on intrinsic clearance of CS in the gastrointestinal tract and liver5,6 and/or differences caused by the effect of the depolymerization process necessary to produce the lower molecular weight, experimental material for this study. Additional studies are required to assess this disparity in the total disaccharide formation after administration of different molecular weight CS. Nonetheless, this study provides the first determination of the bioavailability of CS as indicated by quantifying disaccharides formed by in vitro enzymatic hydrolysis with chondroitinase.

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Table 1. Mean (± SD) Pharmacokinetic Parameters for Total Disaccharides After Oral and Intravenous Administration of 8.0 and 16.9 kDa CS to Horses

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cmax (mcg/ml)</th>
<th>Tmax (hr)</th>
<th>AUC (mcg/ml * hr)</th>
<th>t1/2 (hr)</th>
<th>Fa (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.0 kDa, IV</td>
<td>83.2*</td>
<td>0.083^</td>
<td>36.4*</td>
<td>0.53</td>
<td>—</td>
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<tr>
<td>PO</td>
<td>(0.28)</td>
<td>(0.00)</td>
<td>(0.12)</td>
<td>(0.002)</td>
<td>—</td>
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<tr>
<td>8.0 kDa, IV</td>
<td>4.53**</td>
<td>2.33</td>
<td>11.82**</td>
<td>2.6</td>
<td>32.2</td>
</tr>
<tr>
<td>PO</td>
<td>(0.04)</td>
<td>(0.01)</td>
<td>(0.05)</td>
<td>(0.02)</td>
<td>(9.22)</td>
</tr>
<tr>
<td>16.9 kDa, IV</td>
<td>210*</td>
<td>0.083^</td>
<td>253*</td>
<td>1.2</td>
<td>—</td>
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<tr>
<td>PO</td>
<td>(0.42)</td>
<td>(0.00)</td>
<td>(0.85)</td>
<td>(0.01)</td>
<td>—</td>
</tr>
<tr>
<td>16.9 kDa, PO</td>
<td>36.5**</td>
<td>1.32</td>
<td>54.8**</td>
<td>4.8</td>
<td>22</td>
</tr>
</tbody>
</table>

^1First sampling time point, *p < 0.05, **p < 0.05.
Disaccharide concentrations were determined after treatment of plasma samples with Chondroitinase ABC before HPLC analysis.

References