Metabolism of [3H]pentosan polysulfate sodium (PPS) in healthy human volunteers

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(Received 5 April 2005)

Abstract
Pentosan polysulfate sodium (PPS) is the active ingredient in ELMIRON®, a drug approved for the relief of bladder pain associated with interstitial cystitis. The study objective was to characterize the pharmacokinetic and metabolic profiles of PPS following oral dosing of [3H]PPS. As specific assays for PPS do not exist, metabolic profiling was accomplished through multiple fraction collections and radiochromatographic techniques. Two groups of eight healthy female subjects sequentially received a single oral dose of 200 μCi [3H]PPS supplemented with 300 mg unlabelled PPS or 300 μCi [3H]PPS supplemented with 450 mg unlabelled PPS. Most of the administered dose (84%) was excreted in faeces as intact PPS, and a smaller percentage (6%) was excreted in urine. In summary, orally administered PPS was very poorly absorbed, with the majority of the drug being excreted in faeces as intact PPS and in urine as low molecular weight and desulfated PPS.

Keywords: Pentosan polysulfate sodium, fractionation, desulfated, depolymerized

Introduction
ELMIRON® (pentosan polysulfate sodium, PPS) is a plant-derived, semisynthetic mucopolysaccharide with a xylan backbone that is approved in the USA for the relief of bladder pain or discomfort associated with interstitial cystitis. Interstitial cystitis is a urologic disease characterized by symptoms of urinary urgency, urinary frequency, nocturia, bladder pain and/or discomfort, and characteristic cystoscopic findings. PPS is a heparin-like macromolecule that chemically and structurally resembles glycosaminoglycans. It consists of a polyxylose backbone with one uronic acid residue per nine xylose units. PPS is manufactured from raw materials obtained from beechwood shavings. PPS is a polydisperse
mixture of components of different degrees of sulfation and different chain lengths with MW ranging from 4000 to 6000 daltons. A partial structure of PPS is shown in Figure 1.

Several studies have been conducted to evaluate the pharmacokinetics of PPS in humans. Fellström et al. (1987) measured the plasma and urine concentrations of PPS following intravenous (i.v.) and oral administration in a group of eight healthy subjects using a radioassay. Following i.v. administration of 40 mg PPS, plasma clearance was $49.9 \pm 6.6 \text{ ml min}^{-1}$, of which renal clearance constituted $4.2 \pm 1.2 \text{ ml min}^{-1}$. Only 8% of the i.v. dose was recovered in the urine, which suggests there is extensive metabolism. Following daily oral dosing of 400 mg, steady-state trough plasma concentrations were low (20–50 ng ml$^{-1}$) and the bioavailability was 0.5–1%.

MacGregor et al. (1984) studied the catabolism of an iodinated derivative of pentosan polysulfate. Healthy male subjects received 0.1, 1, 7 or 50 mg $^{125}$I-pentosan polysulfate i.v. or 50 mg subcutaneously. Blood and urine samples collected following dosing were used in a competitive binding assay to measure levels of $^{125}$I-pentosan polysulfate. The half-lives for the 0.1–7-mg doses ranged from 13 to 18 min. At 50 mg i.v., the half-life was 45 min. Tissue distribution studies showed that the majority of labelled material was localized in the liver and spleen. Pentosan polysulfate is desulfated in the liver and spleen and depolymerized in the kidney, and it is likely that the desulfation and depolymerization of pentosan polysulfate is saturable.

Faaij et al. (1999) studied the oral bioavailability of oral PPS in 18 healthy male subjects in a randomized, three-way crossover study. The subjects received an i.v. bolus injection of 50 mg PPS, an oral dose of 1500 mg PPS, or an oral dose of placebo. It was shown that oral bioavailability varied between −0.1 and 0.1% and did not differ from placebo in any respect. Thus, characterization of the metabolism of PPS has been difficult.

The objective of the present study was to characterize the time course of radioactivity in plasma, urine and faeces in humans and to characterize the molecular species in the various biological matrices. In order to increase the chance of obtaining sufficient radioactivity in the circulation, the study was conducted in a sequential manner using two dose levels of PPS.

**Materials and methods**

**Study**

Sixteen healthy adult women (22–56 years) participated in this single-site, single-dose, open-label study. Subjects were divided into two groups of eight subjects each. The study was conducted in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki (1964 and subsequent revisions) and all subjects gave written informed consent.
before participation. Subjects were admitted to the study the day before dosing. Plasma samples were obtained at 0 (predose), 0.25, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 10.0, 12.0, 24.0, 36, 48, 72, 96 and 120 h post-dose. All blood samples were collected into an EDTA-containing Vacutainer™. Plasma samples were centrifuged at about 2500 rpm for about 10–15 min immediately after collection. A 1.5-ml aliquot of plasma sample was placed in a polypropylene tube, which was designated as the primary sample. The remainder of the plasma was placed in another polypropylene tube and was designated as the secondary sample. Urine samples were collected at 0 (predose), 0–4, 4–8, 8–12, 12–24, 24–36, 36–48, 48–72, 72–96 and 96–120 h post-dosing. Faeces samples were collected at 0 (predose), 0–24, 24–48, 48–72, 72–96 and 96–120 h post dosing. Faeces samples were homogenized using a fixed ratio (2:1) of solids to water and then aliquoted into tubes. Plasma, urine and faeces samples were stored at −20°C until analysis.

**Study medication**

In group 1, subjects in the fasted state received a single oral dose of 200 μCi [³H]PPS sodium salt (product Code TRQ 10673; Amersham BioSciences, Cardiff, UK) (<15 mg) supplemented with 300 mg unlabelled PPS formulated in an aqueous solution of 0.9% sodium chloride. Subjects in group 2 received 300 μCi [³H]PPS (<15 mg) supplemented with 450 mg unlabelled PPS formulated in an aqueous solution of 0.9% sodium chloride. The radioactive label had a specific activity of 0.667 mCi mg⁻¹ and a radiochemical purity of 95.6%, with no single major impurity peak.

**Total radioactivity measurement and sample preparation for characterization of PPS metabolites**

Aliquots of urine and plasma samples were analysed by direct liquid scintillation counting (LSC). Aliquots of homogenized faecal samples were solubilized with bleach and then analysed by LSC. Liquid scintillation counting for plasma, urine and other clear samples used Ultima Gold scintillation fluid (PerkinElmer Life and Analytical Sciences, Boston, MA, USA). Solubilized faecal samples were analysed using Hi Ionic Fluor scintillation fluid (PerkinElmer Life and Analytical Sciences). The amount of radioactivity was determined using a Beckman LS6500 liquid scintillation counter (Beckman Instruments, Fullerton, CA, USA).

**Urine.** Frozen urine samples were thawed at room temperature and duplicate aliquots were assayed for total radioactivity. Pooled samples (2% by weight of each time point) from the 0–24 h time points were prepared from three representative subjects from the second group. The pooled urine samples were lyophilized in a FLEXI-DRY freeze-dryer (FTS/Kinetics Thermal Systems, Stone Ridge, NY, USA), and the sublimed liquid (volatile radioactivity) was recovered and analysed for tritium by LSC to determine the portion of tritium present on exchangeable groups. The residue (concentrated urine) was dissolved in a minimal volume of water and fractionated by size-exclusion chromatography high-performance liquid chromatography (SEC HPLC), ion-pairing reversed phase (RP) HPLC, and aminopropyl HPLC.

**Faeces.** A total of six faeces homogenate samples were analysed: two faeces homogenate samples (peak excretion and additional time point) from each of three subjects. Each faeces sample was extracted three times with extraction buffer (150 mM phosphate, pH 7.0,
containing 10% isopropyl alcohol) at a ratio of 5:1 v/w to faeces sample. The extraction was accomplished by shaking vigorously and vortexing intermittently for 5 min, ensuring that a slurry was obtained. Each sample was centrifuged at approximately 27,578 g for 20 min at 4°C (nominal) in a refrigerated centrifuge (Sorvall RC-5, Sorvall, Inc., Newton, CT, USA) between extractions. The extract was separated from the post-extract solid by decanting. These extracts were combined and duplicate aliquots were removed to analyse for total radioactivity by LSC. An aliquot of the combined supernatant was analysed by SEC HPLC and ion-pairing RP HPLC. The radiolabel content of the faeces post-extraction solids (PES) was determined by solubilization. Sodium hypochlorite (household bleach) was added at a ratio of 6 ml bleach g⁻¹ PES. The mixture was placed in a shaker/water bath at 50–55°C overnight. Duplicate aliquots were removed and analysed by LSC. Extraction efficiency was based on the total radioactivity in the original sample and the resulting extract.

**Plasma.** A multi-step fractionating process was used for the pooled plasma samples from the 200- and 300-μCi dosing. Three pooled plasma samples (1-, 1.5- and 2-h samples from 300-μCi dosing; 2-h from 200-μCi dosing; and 3-h from 200-μCi dosing) were fractionated according to the fractionation scheme as far as possible until radioactivity levels in the fractions became indistinguishable from background. Control plasma was spiked with [³H]PPS at the same level and successfully fractionated according to the characterization schemes. In the initial step, plasma was combined with trichloroacetic acid (TCA) and the precipitated protein was pelleted by centrifugation. The supernatant, expected to contain uncharged and low MW metabolites, was counted by LSC, extracted with diethyl ether to remove TCA, and desalted by successive ion exchange chromatography on columns of AG 50W-X8 (to remove cations) and AG 3X-4 (to remove anions). For each step, LSC analysis was done, except for the organic fraction (diethyl ether fraction), which was expected to contain no radioactivity and was discarded without counting. Following desalting, the solution was concentrated by lyophilization. If the concentrated solution had contained sufficient radioactivity, it would have been analysed by SEC HPLC to profile metabolites based on MW. However, the radioactivity in these fractions was too low to be quantitated, so no further analysis was conducted. The sublimed liquid was recovered, pooled, and subsequently analysed for tritium to determine the portion of tritium present as tritiated water.

The TCA-precipitated protein is a highly cationic solid matrix. Intact PPS and sulfated metabolites were therefore expected to co-precipitate as a result of ionic interactions with this solid matrix. The TCA-precipitated protein was extracted with 5 N NaCl to dissociate bound PPS and metabolites, and the extract was desalted on a column of BioGel P-2. The fraction with MW >2000 Daltons, recovered in the excluded or ‘flow-through’ volume of the column, was concentrated by ultrafiltration on a YM-1 membrane (MWCO of 1000) in the presence of excess unlabelled PPS (used as cold carrier to minimize binding of [³H]PPS to the filter and to facilitate recovery of radioactivity during the ultrafiltration step). This high MW fraction was analysed by SEC HPLC and ion-pairing RP HPLC to fractionate/profile PPS metabolites on the basis of MW and degree of sulfation (e.g. net charge), respectively. Combined results from these HPLC characterizations permit assessment of the fraction of plasma radioactivity present as intact PPS.

The fraction recovered from the BioGel P-2 column in the included volume was present in high salt and was therefore not amenable to concentration and subsequent HPLC profiling. This fraction was subjected to ultrafiltration on a YM-1 membrane.
Metabolite analysis by HPLC

Three HPLC methods were employed in the matrix fractionation schemes: SEC HPLC, ion-pairing RP HPLC, and aminopropyl-HPLC. For each, a liquid chromatographic system was assembled that included a controlled volume pumping system capable of generating gradients, a sample injection device, an ultraviolet (UV) detector capable of detection at 254 nm, and a fraction collector capable of accommodating scintillation vials. After injection of urine or faeces extracts, column eluent was collected in 0.5-ml fractions and the fractions were analysed for radioactivity by LSC.

High-performance liquid chromatography systems that consisted of the following equipment were used in the study:

- HPLC pumps: Waters 600 E Multisolvent Delivery System Controller Waters 600 Delivery System Fluid Unit (Millipore Corp., Milford, MA, USA).
- Varian Model 9012 HPLC Pump (Varian Analytical Instruments, Wakefield, CA, USA).
- UV detectors: Waters 486 Tunable Absorbance Detector (Millipore).
- PerkinElmer 785A Programmable Absorbance Detector (PerkinElmer, Shelton, CT, USA).
- Injectors: Waters 717 Autoinjector (Millipore).
- PerkinElmer Series 200LC Autosampler.
- Fraction collectors: Gilson Model FC 204 Fraction Collector (Gilson Medical Electronics, Middleton, WI, USA).
- Software: Turbochrom 6.1.1 (PerkinElmer).

Size exclusion chromatography.

- Column: BioSep SEC-S2000, 5 μm particle size, 7.8 × 300 mm, Guard Column: BioSep SEC-S2000, 5 μm particle size, 7.5 × 75 mm (Phenomenex, Torrance, CA, USA).
- Mobile phase: 10% isopropanol in 150 mM potassium phosphate, pH 7.0.
- Elution: isocratic gradient flow rate at 1 ml min⁻¹; UV detector wavelength 254 nm.

Ion-pairing chromatography.

- Column: Alltima C18, 5 μm particle size, 4.6 × 250 mm (Alltech Associates, Deerfield, IL, USA).
- Mobile phase: buffer A: 20% acetonitrile, 80% 20 mM phosphate buffer (pH 7), 5 mM TBACl (tetrabutyl ammonium chloride).
- Buffer B: 76% acetonitrile, 24% 20 mM phosphate buffer (pH 7), 5 mM TBACl.
- Elution (linear gradient): flow rate at 1 ml min⁻¹; UV detector wavelength 254 nm.
- 0–20 min (0–100% B); 20–30 min (100% B), 30–30.1 min (100 to 0% B), 30.1–40 min, (0% B).

Aminopropyl chromatography.

- Column: Supelcosil LC-NH₂, 5 μm particle size, 4.6 × 250 mm, Guard Column: Supelcosil LC-NH₂, 5 μm particle size, 4.0 × 20 mm (Supelco, Inc., Bellefonte, PA, USA).
Mobile Phase: A: acetonitrile; B: 100 mM ammonium acetate in H2O (pH of approximately 6.8).

Elution (linear gradient): flow rate at 1 ml min\(^{-1}\); UV detector wavelength 254 nm.

0–2 min (0% B), 2–25 min (0–80% B), 25–27 min (80 to 0% B); 27–35 min (0% B).

Results and discussion

The disposition of PPS was studied in two groups of eight healthy female subjects with a mean age of 39.9 years. The first group of subjects received a single oral dose of \([\textsuperscript{3}H]\)PPS (200 mCi, <15 mg) supplemented with 300 mg unlabelled PPS. The second group of healthy female subjects was given a single dose of \([\textsuperscript{3}H]\)PPS (300 mCi, <15 mg) supplemented with 450 mg unlabelled PPS to generate samples containing higher concentrations of radioactivity in the plasma, urine and faeces for metabolic profiling analysis.

Excretion and mass balance in urine and faeces (200-\(\mu\)Ci dose group)

For the 200-\(\mu\)Ci dose group, the total recovery of radioactivity after 120 h was 90.43% ± 8.10% (range 73.22–97.89%). Approximately 6.30% ± 1.11% (range 4.78–7.97%) was excreted in the urine and 84.13% ± 7.71% (range 68.45–91.56%) was recovered in the faeces, indicating the percentage of PPS absorbed was very low. A plot of cumulative excretion of radioactivity in urine and faeces is shown in Figure 2. The level of circulating radioactivity in plasma was very low.

Plasma concentrations of radioactivity

Mean plasma concentrations of radioactivity and key pharmacokinetic parameters are summarized in Table I and shown in Figure 3. PPS recovery of the administered dose in the urine was 6% in each dosing group. Radioactivity counts in plasma samples were insignificant and variable with median (per cent coefficient of variation (CV)) peak plasma...
Table I. Pharmacokinetic parameters for pentosan polysulfate sodium (PPS)-derived radioactivity in plasma.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group 1 200 µCi[^3]^H-PPS + 300 mg PPS (n = 7)</th>
<th>Group 2 300 µCi[^3]^H-PPS + 450 mg PPS (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C\text{max} (ng-eq. ml(^{-1}))</td>
<td>250 (25)</td>
<td>358 (9.3)</td>
</tr>
<tr>
<td>T\text{max} (h)</td>
<td>2.08 (180)</td>
<td>1.83 (250)</td>
</tr>
<tr>
<td>t\text{1/2} (h(^a))</td>
<td>26.5 (23)</td>
<td>19.5 (16)</td>
</tr>
<tr>
<td>AUC\text{0–120} (µg-eq.h.ml(^{-1}))</td>
<td>21.0 (20)</td>
<td>33.8 (13)</td>
</tr>
</tbody>
</table>

Data are medians (coefficient of variation (CV%)).
Plasma concentrations are in ng-eq. ml\(^{-1}\), calculated as disintegrations min\(^{-1}\) (dpm)/specific activity of[^3]^H PPS (1.48 dpm ng\(^{-1}\)).
AUC\text{0–120}, area under the curve from 0 to 120 h; C\text{max}, maximum plasma concentration; t\text{1/2}, half-life; T\text{max}, time to maximum plasma concentration.


Figure 3. Plasma radioactivity concentrations (mean ± SD) after a single dose of[^3]^H PPS (n = 8).

PPS concentrations of 250 (25) ng-eq. ml\(^{-1}\) for group 1 and 358 (9.3) ng-eq. ml\(^{-1}\) for group 2. These peak plasma concentrations in groups 1 and 2 were seen at similar time points, 2.08 and 1.83 h, respectively. The elimination half-lives, estimated from the urinary excretion data, were similar in the two dose groups.

**Metabolic profiling analysis**

Samples of urine, faeces and plasma were chosen from three selected subjects in the higher dose group for metabolic profiling analysis using validated analysis methods to determine the change in metabolic profile (if any) with time. In addition, a pooled urine sample from the 0–24-h time points was prepared from each of the same three subjects to determine the overall percentages of each metabolite region as a percentage of administered dose.
Urine. Urine samples were lyophilized, and the sublimed liquid was recovered and subsequently analysed for tritium to determine the portion of tritium present on exchangeable groups. The residue was dissolved in a minimal volume of water and fractionated by SEC HPLC, ion-pairing RP HPLC, and aminopropyl HPLC. Profiles obtained by SEC reflect changes in the MW distribution of PPS, and can be interpreted as depolymerization of drug substance. Profiles obtained by ion-pairing RP HPLC reflect changes in the net charge distribution of PPS, and can be interpreted as desulfation of drug substance. Profiles obtained by aminopropyl-HPLC reflect the degree of heterogeneity in low-MW urinary metabolites of PPS, and indicate the possible number of distinct low-MW metabolites formed from PPS. Representative SEC, ion-pairing RP HPLC, and aminopropyl HPLC chromatograms of pooled urine samples are shown in Figure 4.

For the 0–24-h pooled urine samples, volatile radioactivity was minimal (0.08% ± 0.03% administered dose). Low levels of intact PPS were observed (0.14% ± 0.06% of administered dose) based on results from ion-pairing HPLC analysis. The SEC HPLC analysis indicated that radioactivity corresponding to PPS in retention time corresponded to 1.03% ± 0.70% of the administered dose, and this radioactivity consisted of intact PPS and desulfated PPS. Based on the difference between the SEC and ion-pairing RP HPLC analysis, desulfated PPS corresponded to 0.89% ± 0.64% of the administered dose (desulfated PPS was >61% desulfated compared with intact PPS based on ion-pairing RP HPLC of sulfation standards prepared during the validation study). The majority of radioactivity excreted in the 0–24-h pooled urine samples was of lower MW than PPS (3.86% ± 0.08% administered dose based on SEC HPLC analysis).

The aminopropyl chromatography of urine showed at least six regions of radioactivity. The major lower MW peak (region C, 3.62% ± 1.15% administered dose) was isolated from aminopropyl chromatography and further characterized by ultrafiltration using a 3000 MW cut-off filter; these methods indicated this peak contained a mixture of components of lower MW than PPS. The metabolic profiling with urine indicates that PPS was metabolized extensively by desulfation and depolymerization. The HPLC profiling undertaken with urine samples from selected time points showed similar HPLC profiles at the various time points (0–4, 8–12 and 24–36 h after dosing), indicating the metabolic profile did not change appreciably with time.

Faeces. Faeces samples from selected time points were extracted and the extracts were analysed by both SEC and ion-pairing RP HPLC to determine the degree of depolymerization and desulfation of the drug substance. The data indicate that the faeces-contained radioactivity consists predominantly of unchanged PPS. The level of PPS accounted for 52.26% ± 9.14% of the administered dose, based on results from ion-pairing HPLC analysis of the faecal-contained radioactivity in the samples that were analysed.

Plasma. Levels of radioactivity in plasma were very low (about 500 dpm g⁻¹ plasma at Cmax). The recovery of plasma-contained radioactivity in the TCA supernatant was 57.96% for the 1-, 1.5- and 2-h pooled samples from the 300-μCi dosing, 66.63% for the 2-h pooled sample from the 200-μCi dosing, and 58.39% for the 3-h pooled sample from the 200-μCi dosing. The radioactivity in the TCA supernatant contained both volatile radioactivity (17.42% for the 1-, 1.5- and 2-h pooled samples from the 300-μCi dosing, 22.83% for the 2-h pooled sample from the 200-μCi dosing, 3-h sample not analysed) and PPS metabolites; the radioactivity level in the metabolite-containing fractions (after ion-exchange columns) was too low for HPLC profiling. The recovery in the NaCl extract of the protein pellet was 21.44% for the 1-, 1.5- and 2-h pooled samples from the 300-μCi dosing,
Figure 4. Pentosan polysulfate sodium (PPS) and metabolites in pooled urine 0–24 h after a single 450-mg oral dose of [3H]PPS; size-exclusion chromatography (top), ion-pairing HPLC (middle) and aminopropyl chromatography (bottom).
5.16% for the 2-h pooled sample from the 200-μCi dosing, and 16.96% for the 3-h pooled sample from the 200-μCi dosing; the level of radioactivity in the NaCl extract was too low for subsequent analysis and confirmation of the radioactivity in this fraction as PPS. This radioactivity can be considered as ‘PPS-like,’ and would correspond to approximately 70 ng g⁻¹ plasma for the 1-, 1.5- and 2-h pooled samples from the 300-μCi dosing. This Cmax level for PPS in plasma is similar to that observed in other reported studies (Marshall et al. 1997), where Cmax values of about 70 ng ml⁻¹ were seen after a single oral dose (400 mg m⁻² = 780 mg) of PPS.

The results obtained in humans are similar to those obtained in rats. Odlind et al. (1987) reported a preferential localization of PPS in the urinary tract of rats after oral administration of [3H]PPS. In a metabolic profiling study we conducted in rats dosed with [3H]PPS, a similar profile of metabolic disposition of PPS was observed (6.9% was excreted in urine, 84.4% in faeces and 0.06% in plasma). Radioactivity excreted in urine comprised mostly of components of lower MW than PPS. The degree of desulfation of PPS in urine was less than that found in humans as seen in the ion-pair chromatography. Radioactivity in faeces consisted mostly of unabsorbed PPS. Radioactivity in plasma was characterized more extensively than was done for the plasma samples from human plasma due to higher radioactivity levels. Intact PPS represented only a small fraction (<1%) of the plasma-contained radioactivity (data not shown).

In summary, PPS was poorly absorbed (bioavailability less than 1%). Unabsorbed PPS was excreted predominantly unchanged in the faeces. Radioactivity in the urine consisted mostly of components of lower MW and lower degrees of sulfation than PPS, indicating PPS was metabolized by depolymerization and desulfation. Kirkman et al. (1994) observed similar metabolic pathways in low molecular weight heparin. Radioactivity circulating in plasma was very low, precluding extensive metabolic profiling experiments.

Acknowledgement

The study was funded by ALZA Corporation.

References