Studies on the Metabolic Fate of Sodium Hyaluronate (SL-1010) after Intra-articular Administration II: Distribution, Metabolism and Excretion after Repeated Intra-articular Injection into Rabbit Knee

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Key words: Sodium hyaluronate (SL-1010), Repeated intra-articular administration, Rabbit, Distribution, Excretion, Metabolism

Summary
A 1% 14C-SL-1010 solution (3mg/kg) was administered repeatedly into the cavitas articulare of left rabbit knee at 4 day intervals for a total of 7 times.
1. Residual radioactivity in synovial fluid was approximately 60% of each dose at 24hrs after the 4th and 7th administrations and was comparable to that observed after the first administration. The clearance of radioactivity from synovial fluid observed after the 7th administration was comparable to a simulated curve projected from results for the first administration study group, and no accumulation was observed as a result of repeated administration.
2. Plasma levels of radioactivity reached the steady state following the 3rd administration and gradually decreased from 5 days after the 7th administration with an elimination half-life of 106.0hrs. The observed plasma profile of radioactivity was similar to that simulated from results of the single dose study.
3. The primary route of excretion was the expired air. The cumulative excretion of radioactivity in expired air reached the steady state after the 6th administration and reached 81.5% of the total dose at 14 days after the 7th administration. The cumulative excretion of radioactivity in urine and feces were 3.3% and 2.9%, respectively. These results were similar to those observed in the single dose study.
4. The levels of radioactivity in various tissues surrounding the dosing side of the knee joint gradually increased with repeated administration. At 24hrs after the 4th administration, the level had risen to 0.9-2.8 times higher levels of that at 24hrs following the first administration, and the peak concentration observed after the 7th administration was 1.4-3.2 times higher than the level at 24hrs following the first administration. The level of radioactivity was highest in the synovial tissue and was followed in order of decreasing radioactivity by the infrapatellar fat pad, meniscus, patellar ligament, cartilage and patella.
5. The level of radioactivity in the principal tissues beyond the knee joint tended to increase gradually with repeated administration, reaching a peak at 24hrs following the 7th administration. The peak values for liver, lung, kidney, spleen, Harderian gland, adrenal gland and heart were 2.6-13.6 times higher than the levels at 24hrs after the first administration. At 14 days after the 7th administration, these concentrations had decreased to 10-60% of peak values.
Introduction

SL-1010 is a highly purified, high molecular weight sodium hyaluronate synthesized via *Streptococcus zooepidemicus* in Shiseido Co., Ltd. This compound is currently under development as a drug for the treatment of osteoarthritis and periarthritis scapulohumeralis.

The pharmacokinetic study of SL-1010 (distribution, metabolism and excretion) was reported in a preceding paper presenting the results of a single intra-articular administration of 14C-labeled SL-1010 (14C-SL-1010) into the rabbit knee\(^2\).

Prior to the present study, a preliminary study was conducted to determine the dosing schedule using 14C-SL-1010, and in the present study, the pharmacokinetics of SL-1010 after repeated administration was investigated.

Materials and Methods

1. Labeled compound

14C-SL-1010 was synthesized from N-acetyl-D-[1-14C]glucosamine (Amersham, England; specific radioactivity, 2.17GBq/m mole; radiochemical purity, at least 97%) as a precursor via *Streptococcus zooepidemicus*\(^2\) and purified.

The limiting viscosity (\(\eta_1\)), viscosity-average molecular weight and specific radioactivity of 14C-SL-1010 were respectively 29.9g/dl, 2030kDa and 80.4kBq/mg in Lot No. H-14, 29.1, 1960 and 67.9 in Lot No. H-16, 29.7, 2010 and 52.5 in Lot No. H-18, and 30.3, 2040 and 40.0 in Lot No. H-19. The radiochemical purity of the compound was confirmed to be 98.5% or higher using the method described in the preceding report\(^2\). Each lot of this compound was delivered from Shiseido Co., Ltd. as a 1% (w/v) 14C-SL-1010 isotonic phosphate-buffered solution (pH7.3).

2. Experimental animals

Male Japanese albino rabbits (body weight 2-3kg, Sankyo Labo Service Co., Japan) were fed pellet feed (CR-3, Clea Japan Inc., Japan) and tap water *ad libitum*. After acclimation to a temperature of 22±1°C and a humidity of 60±10%, animals were submitted to the study in groups of three.

3. Dosage and method of administration

After removing hair at the knee joint, the animals were administered 3mg/kg of a 1% 14C-SL-1010 solution into the cavitas articulare of the left knee as described in the preceding paper\(^2\). A total of 7 administrations were performed at 4 day intervals.

The lot Nos. H-14, H-18 and H-19 solutions of 14C-SL-1010 were used for the measurements of radioactive concentration in synovial fluid and tissues, and the Lot No. H-16 solution was used for the measurement of blood and plasma radioactive concentrations and excretion of radioactivity in expired air, urine and feces.

4. Measurement of radioactivity in synovial fluid

Following the administration of 14C-SL-1010, animals were killed by exsanguination
from the posterior aorta under ether anesthesia. Then, 1 ml of physiological saline was injected into the cavitas articularis and the synovial fluid was collected after bending the knee 20 times. Sampling points were at 24hrs and 2, 3 and 4 days after the first administration, 24hrs after the 4th administration and 2hrs, 24hrs, 6 and 14 days after the 7th administration.

5. Measurement of radioactive concentration in blood and plasma

Following the administration of $^{14}$C-SL-1010, a 500μl of blood sample was collected from the auricular vein according to the time schedule. Sampling points were set at 24hrs after the first administration to 14 days after the 7th administration, with 24hr intervals. From the blood sample obtained, 200μl was dried on ashless filter paper (Toyo Roshi, Inc., Japan), and used for the measurement. After conventional centrifugation of the remaining blood sample, plasma was obtained and used for the measurement.

6. Measurement of excretion of radioactivity in expired air, urine and feces

Animals were housed in metabolic cages after administration of $^{14}$C-SL-1010. The naturally-excreted urine and feces were collected and used for radioactive measurement. Expired air was absorbed as $^{14}$CO₂ into an absorbent containing primarily monoethanolamine, and measured for radioactivity. Sampling points were set at 24hrs after the first administration through 14 days after the 7th administration, at 24hr intervals.

7. Measurement of radioactive concentration in tissues

Following the administration of $^{14}$C-SL-1010, animals were killed by exsanguination from the posterior aorta under ether anesthesia. After synovial fluid was obtained as described above, synovial tissue was obtained as described in the previous report 3, followed by the patellar ligament, meniscus, articular cartilage, patella, infrapatellar fat pad, femoral muscle, thigh bone and femoral bone marrow. In respect to systemic organs, the Harderian gland, heart, lung, liver, spleen, kidney and adrenal gland were collected and measured for wet weight. All of the light organs and part of the others were homogenized for measurement of radioactivity. Sampling times were 24hrs after the first and 4th administrations and 2hrs, 24hrs, 6 and 14 days after the 7th administration.

8. Measurement of radioactivity

Blood and fecal samples were prepared by combustion in an automatic combustion system (Aloka ACS-112). Toluene scintillator was added to the absorbed expired air. Other samples were solubilized using an alkaline solubilizing agent (primarily potassium hydroxide) and decolorized with an aqueous hydrogen peroxide solution, if necessary, and dioxane scintillator was added to prepare samples for measurement. Radioactivity of each sample was measured with a liquid scintillation counter (Aloka LSC-1000) for 10 minutes.
9. Pharmacokinetic analysis

Synovial fluid: The profile of elimination of radioactivity from synovial fluid after the first administration was closely fitted by an equation of first order. Therefore, the simulation curve was calculated by a one compartment model of the bolus injection using nonlinear least-squares analysis (simplex method).

Blood and plasma: In a single dose study, the levels of radioactivity in blood and plasma were measured until 9 days after administration. On and after the last sampling time, those levels were calculated from the slope of elimination phase using the least-squares method with extrapolating to 14 days. Based on these results, the simulation curves of 7 repeated administration at 4 day intervals were calculated by overlying principle. The maximum concentration (C_max) and its time of occurrence (T_max) were read directly from the concentration-time data. Apparent half-life (T_1/2) was estimated using the least-squares method based on the concentration-time data. The area under the concentration-time curve (AUC) was calculated by trapezoidal rule.

Results

1. Elimination of radioactivity from synovial fluid

Fig. 1 shows the residual radioactivity in synovial fluid at 24hrs and 2, 3 and 4 days (just prior to the second administration) after the first administration, 24hrs after the 4th administration and 2hrs, 24hrs, 6 and 14 days after the 7th administration and a

![Graph](image-url)

Fig. 1 Elimination profile of radioactivity from synovial fluid after repeated intra-articular administration of 14C-SL-1010 3mg/kg at four day intervals to male rabbits. Each point represents the mean ± S.D. for three animals. Arrows mark the dosings.

- - - : simulated curve,  • : measured value.
simulated curve projected from results obtained from the first administration study group.

Residual radioactivities in synovial fluid at 24hrs after the 4th and 7th administration were approximately 60% of each dosage, and were comparable to that at 24hrs after the first administration. In addition, the profile of elimination of radioactivity from synovial fluid observed after the 7th administration was similar to that of the simulated curve, and no accumulation in the cavitas articulare was observed.

2. Blood and plasma concentrations

Fig. 2 shows the levels of radioactivity in blood and plasma during repeated administration and up to 14 days after the 7th administration and a simulated curve projected from results of the single dose study.

Blood levels of radioactivity reached the steady state (approximately 1µg equiv./ml) following the 4th administration and gradually decreased from 7 days after the 7th administration with T1/2 of 131.0hrs.

Plasma levels of radioactivity reached the steady state (0.66-0.75µg equiv./ml) following...
the 3rd administration and gradually decreased from 5 days after the 7th administration with $T_{1/2}$ of 106.0hrs.

The levels of radioactivity in blood and plasma were slightly lower than the corresponding simulated values during the period of repeated administration. However, the actual values were then higher than the simulated curves on and after 6 or 7 days after the last administration.

3. Excretion in expired air, urine and feces

Fig. 3 shows the cumulative excretion of radioactivity in expired air, urine and feces during repeated administration and up to 14 days after the 7th administration.

The primary route of excretion was expired air. The cumulative excretion of radioactivity in expired air reached the steady state following the 6th administration, and 81.5 ±1.2% of the total dose was excreted by 14 days after the 7th administration. Cumulative excretion of radioactivity in urine and feces were 3.3±0.6 and 2.9±0.2%, respectively, showing the minor contribution for these excretion routes.

The radioactivity in the carcass was 8.9±1.0% of the total dose at 14 days after the 7th administration.
Table 1  Tissue distribution of radioactivity after repeated intra-articular administration of $^{14}$C-SL-1010 3mg/kg at four day intervals to male rabbits.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Concentration of radioactivity (µg equiv. of SL-1010/g or ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>At 24hr after last administration</td>
</tr>
<tr>
<td></td>
<td>1st</td>
</tr>
<tr>
<td>Blood</td>
<td>0.14 ± 0.01</td>
</tr>
<tr>
<td>Plasma</td>
<td>0.26 ± 0.02</td>
</tr>
<tr>
<td>Harderian gland</td>
<td>1.08 ± 0.22</td>
</tr>
<tr>
<td>Heart</td>
<td>0.13 ± 0.01</td>
</tr>
<tr>
<td>Lung</td>
<td>0.22 ± 0.06</td>
</tr>
<tr>
<td>Liver</td>
<td>1.14 ± 0.08</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.25 ± 0.01</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.27 ± 0.06</td>
</tr>
<tr>
<td>Adrenal</td>
<td>0.27 ± 0.05</td>
</tr>
<tr>
<td>Synovial tissue (Injected site)</td>
<td>386.59 ± 93.49</td>
</tr>
<tr>
<td>Ligament</td>
<td>38.90 ± 12.87</td>
</tr>
<tr>
<td>Meniscus</td>
<td>140.30 ± 39.87</td>
</tr>
<tr>
<td>Articular cartilage</td>
<td>41.19 ± 7.91</td>
</tr>
<tr>
<td>Patella</td>
<td>24.47 ± 1.69</td>
</tr>
<tr>
<td>Fat pad</td>
<td>120.84 ± 53.84</td>
</tr>
<tr>
<td>Femoral muscle</td>
<td>0.34 ± 0.07</td>
</tr>
<tr>
<td>Thigh bone</td>
<td>0.29 ± 0.05</td>
</tr>
<tr>
<td>Femoral bone marrow (noninjected site)</td>
<td>0.79 ± 0.04</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.D. for three animals.
4. Tissue concentrations

Table I shows the levels of radioactivity in tissues at 24hrs after the first and 4th administration, and at 2hrs, 24hrs, 6 and 14 days after the 7th administration.

The levels of radioactivity in various tissues of the dosing side of the knee joint gradually increased with repeated administrations. At 24hrs after the 4th administration, the level had risen to 0.9-2.8 times higher levels of that at 24hrs following the first administration, and, in addition, the peak concentration observed after the 7th administration was 1.4-3.2 times higher than the level at 24hrs following the first administration. The level of radioactivity was highest in the synovial tissue and was followed in order of decreasing radioactivity by the infrapatellar fat pad, meniscus, patellar ligament, cartilage and patella. At 14 days after the 7th administration, these radioactive concentrations were decreased to a level lower than that observed at 24hrs after the first administration. On the other hand, tissues of the non-dosing side of the knee joint showed extremely low radioactive concentrations compared with those of the dosing site.

The levels of radioactivity in the systemic organs beyond knee joint also showed a tendency to increase gradually with repeated administrations, reaching a peak at 2 or 24hrs after the 7th administration. The peak values for liver, lung, kidney, spleen, Harderian gland, adrenal gland and heart were 2.6-13.5 times higher than the levels at 24hrs after the first administration. By 14 days after the 7th administration, the concentrations had decreased to 10-60% of those at peak.

Discussion

A study of sodium hyaluronate after repeated intra-articular administration has been reported by Sakamoto et al. The dosing schedule was a combination of 3 and 4 day intervals, and day 21 was the final dosing day. Previous to the present study, a preliminary study was conducted to evaluate the pharmacokinetics of sodium hyaluronate after a single intra-articular administration at the doses of 3mg/kg and 6mg/kg (3mg/kg for each knee joint). The results showed increases in Cmax and AUC0–∞ proportionally, but no difference was observed in Tmax and T1/2 (Table II). The excretion of radioactivity in expired air, urine and feces were 75, 3 and 2%, respectively, with no difference observed between the two doses. Moreover, the residual radioactivity in synovial fluid after intra-

Table II Pharmacokinetic parameters of radioactivity in blood and plasma after intra-articular administration of 14C-SL-1010 to male rabbits.

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>3</th>
<th>6a)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>blood</td>
<td>plasma</td>
</tr>
<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt; (hr)</td>
<td>120± 0</td>
<td>96± 0</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (μg equiv./ml)</td>
<td>0.48± 0.04</td>
<td>0.42± 0.05</td>
</tr>
<tr>
<td>T&lt;sub&gt;1/2&lt;/sub&gt; (hr)</td>
<td>109.2±15.1</td>
<td>87.7±11.3</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0–∞&lt;/sub&gt; (μg equiv.·hr/ml)</td>
<td>96.1± 4.9</td>
<td>89.1± 5.5</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.D. for three animals.
a) Administered to both knee joints at 3mg/kg/joint.
articular administration at the dose of 3mg/kg showed a similar profile to that of 1mg/kg reported in the preceding study\(^2\). The elimination profile of radioactivity from synovial fluid after administration of 3mg/kg was closely fitted by an equation of first order. \(T_{1/2}\) was 30hrs, and almost no residual radioactivity in synovial fluid was observed at 7 days after administration. Based on these results, a dosing schedule with 3mg/kg delivered at 4 day intervals was selected. On the other hand, five dosings at 1 week intervals are considered likely in the clinical use of SL-1010. Accordingly, 7 repeated administrations were chosen in the present study to cover the dosing period expected for clinical use.

Residual radioactivity observed in synovial fluid at 24hrs after the 4th and 7th administration were comparable to that observed at 24hrs after the first administration. The radioactive clearance from the cavitas articularis observed after the 7th administration was also comparable with the simulated curve. It was therefore confirmed that there was no residual accumulation observed as a result of repeated administration.

In the cavitas articularis, the tissues contacting directly to synovial fluid such as synovial tissue, meniscus, and cartilage showed a gradual increase with repeated administrations. However, the peak observed following the 7th administration was at most twice that of the first administration, and the elimination observed was almost comparable to that for single dosing reported in the preceding paper\(^2\). Thus, it is considered that there is no accumulation as a result of repeated administration. According to the preceding paper\(^2\), intra-articularly administered \(^{14}\)C-SL-1010 has been reported not to be metabolized in the cavitas articularis, but rather metabolized primarily in lumb-iliac and popliteal lymph nodes following a molecular weight-related transmigration to the lymph nodes, and is then distributed to the systemic circulation in the form of the low molecular weight metabolite. These results suggest that the synovial tissue play an important role in molecular weight-related transmigration from the cavitas articularis to lymph nodes.

The levels of radioactivity in blood and plasma were slightly lower than the corresponding simulated values during the period of repeated administration. However, this phenomenon reversed on and after 6 or 7 days after the last administration. With regard to radioactivity in plasma and blood cells during and after repeated administration, the amount of \(^{14}\)C-hyaluronic acid was found to be as low as 2.1% or less, and 55.9–93.1% was present in the plasma and blood cells protein fractions\(^5\). No difference was observed from the preceding single dose study\(^2\). On the basis of these results, it is concluded that there is no accumulation of \(^{14}\)C-hyaluronic acid in blood as a result of repeated administration.

The level of radioactivity in the principal tissues beyond the knee joint tended to increase gradually with repeated administration, reaching a peak at 24hrs following the 7th administration. At peak value, a pattern of distribution similar to that in the preceding single dose study\(^2\) was noted. The levels of radioactivity observed in the principal tissues at 24hrs after the 7th administration were 2.6–13.5 times higher than the levels at 24hrs after the first administration. In respect to radioactive compounds in the liver, kidney and spleen, as low as 2.9% or less of the radioactivity was delivered from \(^{14}\)C-hyaluronic acid while 49.1–78.7% was obtained from the protein fraction\(^5\), in agreement with the
result obtained from the analysis of protein fraction in liver in the preceding single dose study. These results indicate that the radioactivity observed in major organs was mainly derived from biomolecules such as protein incorporating degraded 14C-hyaluronate and there is no accumulation resulting from repeated administration of 14C-hyaluronate.

The primary route of excretion observed with repeated administration was expired air. Total excretions in expired air, urine and feces were 81.5, 3.3 and 2.9%, respectively. These proportions of excretion were similar to those obtained in the preliminary single dose study. The radioactivity in the carcass was 8.9% of the total dose at 14 days after the 7th administration, it seems likely that the radioactivity in the carcass was mainly derived from biomolecules such as protein incorporating degraded 14C-hyaluronate.

Thus, no accumulation resulting from repeated intra-articular administration of SL-1010 to rabbits was observed in synovial fluid, blood, plasma, tissues of knee joint or principal organs, and no difference was observed in the excretion profile for expired air, urine and feces compared with the single dose study. We therefore conclude that repeated administration of this drug has no influence on its metabolic fate.

References
5) Maruho Co., Ltd., unpublished.