Estimation of joint fluid volume in the knee joint of rabbits by measuring the endogenous calcium concentration

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Key words: Joint fluid volume, calcium, arthritis, rabbit.

ABSTRACT

Objectives
The purpose of this study was to develop a new, simple, and more efficient method for estimating joint fluid volume in the knee of experimental animals by measuring endogenous calcium concentration, which is maintained rigidly at a constant level in the body fluid.

Methods
Calcium concentrations in the plasma and joint cavity lavage were colorimetrically measured in normal and papain-induced arthritic rabbits. The joint fluid volume was estimated by dividing the total calcium concentration in the joint cavity lavage (µg/joint) by calcium concentration in the plasma (µg/ml). To confirm the relevancy of this method, radioactivity in the plasma and joint cavity lavage was determined after intravenously injecting ¹⁸⁶H₂O. The correlation between the joint fluid volumes obtained from the two different methods was examined to evaluate the validity of the method involving measurement of the calcium concentration.

Results
The joint fluid volumes of normal rabbits were estimated at 401 µl/joint and that in the arthritic rabbits at 680 µl/joint by measuring calcium concentration, respectively. These values were not significantly different from those estimated by radioactivity in the plasma and joint cavity lavage (normal: 425 µl/joint, arthritis: 761 µl/joint). A statistically significant correlation was observed between the values obtained from the two methods (r = 0.985).

Conclusion
This method is considered useful for the evaluation of therapeutic medicine for arthritis or for hydraphrosis research using experimental animal models.

Introduction
In various joint diseases such as osteoarthritis, rheumatoid arthritis, or traumatic arthritis, hydraphrosis is generally observed (1, 2). Hydraphrosis is well known to result from an increase in the vascular permeability causing the inflammation of synovial membrane. The subjective symptom is discomfort by the joint swelling due to the increase of effusion into the joint cavity, and the range of motion in the joint is limited. Also, in several experimentally induced arthritis models the joint fluid is retained in the joint cavity (3, 4). Therefore, the volume of joint fluid is considered a useful marker for the evaluation of therapeutic medicine for arthritis. Dilution methods reportedly can estimate the joint fluid volumes of guinea pigs and rabbits (5, 6). In these methods, a molecule marker such as hydroxyethyl-starch or FITC-labeled dextran with a high molecular weight is injected into the joint cavity. After the marker molecule mix with the joint fluid, a constant volume of the diluted joint fluid is recovered. The joint fluid volume is able to estimate by measuring ratio of marker molecule. Although these methods are simple, efficient, and useful for estimating the joint fluid volumes of small laboratory animals, an exogenous marker molecule with a high molecular weight is required. In addition, an optimum injection volume of the maker molecule must be decided according to each specific experimental animal. In this study, we focused on the calcium concentration, present at a constant level in the body fluid, to estimate the joint fluid volume, and then investigated a method using endogenous calcium concentrations in the plasma and joint cavity lavage. Furthermore, radioacti-
Table II. Ca concentrations in the plasma and original joint fluid.

<table>
<thead>
<tr>
<th></th>
<th>Plasma (µg/ml)</th>
<th>Original joint fluid (µg/ml)</th>
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<tbody>
<tr>
<td>Normal rabbit</td>
<td>127 ± 9.53</td>
<td>121 ± 4.22</td>
</tr>
<tr>
<td>Arthritic rabbit</td>
<td>124 ± 5.96*</td>
<td>119 ± 3.90*</td>
</tr>
</tbody>
</table>

The concentrations in the plasma and original joint fluid represent the mean ± S.D. of 6 rabbits and 12 joints, respectively. Ca concentrations in the plasma and original joint fluid of normal rabbits compared with those of arthritic rabbits.

*Not significant by Student’s t-test.

The concentration in the plasma and joint fluid lavage was determined after an intravenous injection of \(^{3}\text{H}_2\text{O}\) to estimate the joint fluid volume. The correlation between the values of the joint fluid volumes obtained by these two methods was examined, and the validity of the method involving measurement of calcium concentration was evaluated.

**Materials and methods**

**Experimental animals**

Female Japanese white rabbits weighing 2.7 ± 3.3 kg were used in this study. The research protocol used for these experiments was approved by the Animal Research and Care Committee at the author’s institute. The calcium concentrations in the plasma and original joint fluid were determined in 12 rabbits (12 joints of 6 normal and 12 joints of 6 arthritic). For the estimation of joint fluid volume, the calcium concentrations and radioactivities in the plasma and joint cavity lavage were measured in 6 rabbits (6 joints of 3 normal and 6 joints of 3 arthritic). As a separate examination, \(^{3}\text{H}_2\text{O}\) was intravenously administered to 6 normal rabbits in order to determine an appropriate measurement time point in the study estimating joint fluid volume based on radioactivity (3 animals/measurement point). The distribution of animals and measurement items are listed in Table I.

**Arthritis induced by an intraarticular injection of papain**

Papain powder (Sigma Chemical Co., St Louis, MO, USA) was dissolved in physiological saline containing 0.8 mg/ml of L-systeine at a concentration of 10 mg/ml. The solution was filtered through a membrane with a pore size of 0.45 µm, and 0.15 ml of the filtrate was injected with a 27-gauge injection needle into each joint cavity of both rabbit knees. The administered dose of papain was 1.5 mg/joint. Rabbits receiving papain were used as arthritic models one day later.

**Sampling of plasma**

Blood was taken from an artery at the time of animal sacrifice and heparinized. The plasma was then separated by centrifugation at 1,800xg for 15 minutes.

**Sampling of original joint fluids**

The animals were sacrificed by means of an intravenous excess dose of pentobarbital (Nembutal, Dainabot Laboratories, USA), and both knee joints were separated. In the case of arthritic joint fluid, the original joint fluid was removed from the joint cavity via a plastic syringe with a 25-gauge injection needle and centrifuged at 1,800 xg for 15 minutes. The resulting supernatant was used as the original joint fluid of arthritic rabbits.

Using an accurately weighed dry piece of filter paper (5 mm x 3 mm), the normal original joint fluid was recovered. The piece of filter paper was inserted into the joint cavity and left for 30 seconds. Once removed from the joint cavity, the paper was weighed using a microbalance. The weight of original joint fluid was calculated from the difference between the wet dry weights of the piece of filter paper and then divided by the specific gravity of normal rabbit joint fluid (1.02) (8).

**Recovery of the joint cavity lavage**

After both knee joints were separated at the time of sacrifice, 2 ml of physiological saline was injected into the joint cavity via a plastic syringe with a 25-gauge injection needle and mixed with the joint fluid by repeated flexing, 50 times. The total volume of the joint cavity was removed. This procedure was repeated 3 times. The volume of lavage recovered was accurately measured and stored at –20°C until analysis.

**Determination of calcium concentration**

Calcium concentrations in the plasma and joint fluid were colorimetrically determined (Calcium C-Test Wako Kit, Wako Pure Chemical Industries Ltd., Osaka, Japan) using the method of Gitelman et al. (9).

**Measurement of radioactivity**

One ml of saline solution containing 100 µCi of \(^{3}\text{H}_2\text{O}\) (ICN, Japan) was injected into the auricular veins of 6 normal rabbits. After 1 hour, 3 rabbits were sacrificed, and after 3 hours the remaining 3 rabbits were sacrificed. The plasma and original joint fluid were collected according to the method described above. The piece of filter paper in which 100 µl of the plasma or the joint fluid was absorbed was placed in a scintillation vial and 10ml of emulsifying scintillator was added. After vigorous shaking, the radioactivity in the plasma and original joint fluid 1 and 3 hours after the time of \(^{3}\text{H}_2\text{O}\) injection was measured using a liquid scintillation counter (LSC-700, Aloka, Tokyo, Japan).

**Estimation of the joint fluid volume by measuring calcium concentration**

An intravenous injection of \(^{3}\text{H}_2\text{O}\) was administered to 6 rabbits (6 joints of 3 normal and 6 joints of 3 arthritic) at a dose of 100 µCi/rabbit. After 3 hours, all animals were sacrificed and the plasma and joint cavity lavage were collected. Calcium concentrations of the collected plasma and joint fluid lavage were determined and the joint fluid volume (µg/joint) was estimated from the following formula:

\[
\text{Total amount of calcium in joint cavity lavage (µg/joint)} \times \frac{100}{\text{Ca concentration in plasma (g/ml)}}
\]

**Estimation of the joint fluid volume by measuring \(^{3}\text{H}_2\text{O}\) radioactivity**

Radioactivity in the collected plasma and joint cavity lavage was determined and the joint fluid (µg/joint) was estimated from the following formula:

\[
\text{Total amount of radioactivity in joint cavity lavage (dpm/joint)} \times \frac{1000}{\text{radioactivity in the plasma (dpm/ml)}}
\]
Table III. Radioactivity in the plasma and original joint fluid after an intravenous injection of $^3$H$_2$O.

<table>
<thead>
<tr>
<th>Sampling time (h)</th>
<th>Sample</th>
<th>Radioactivity (x10$^3$ dpm/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Plasma</td>
<td>183 ± 6.00</td>
</tr>
<tr>
<td></td>
<td>Joint fluid</td>
<td>151 ± 21.0 *</td>
</tr>
<tr>
<td>3</td>
<td>Plasma</td>
<td>187 ± 19.0</td>
</tr>
<tr>
<td></td>
<td>Joint fluid</td>
<td>165 ± 9.60</td>
</tr>
</tbody>
</table>

The radioactivity in the plasma and original joint fluid represents the mean ± S.D. of 3 rabbits and 6 joints, respectively.

*p < 0.05 versus plasma, by Student’s t-test.

Statistical analysis

Statistical analysis of the following data was carried out using the Student’s t-test: 1) test for the differences between the data from normal and arthritic rabbits, 2) test for the difference between the radioactivity in plasma and that in the original joint fluid after intravenous administration of $^3$H$_2$O, and 3) test for the difference between the joint fluid volume estimated using calcium concentrations and that using radioactivities. A linear correlation coefficient between the joint fluid volumes obtained from the two methods was calculated, and the correlation was examined using the r-table.

Results

Calcium concentrations in the plasma and original joint fluid (Table II)

Calcium concentrations in the plasma and original joint fluid of the arthritic rabbits were similar to those obtained from the normal rabbits. In both the normal and arthritic rabbits, calcium concentrations in the original joint fluids were slightly lower than those in the plasma.

Radioactivity in the plasma and original joint fluid after intravenous injection of $^3$H$_2$O (Table III)

The plasma and original joint fluid were collected from normal rabbits 1 and 3 hours after the intravenous injection of $^3$H$_2$O. Radioactivity in the original joint fluid was significantly lower than that in the plasma 1 hour after $^3$H$_2$O injection, but no significant difference in the radioactivity of these samples was observed 3 hours later. Therefore, the joint fluid volume was estimated by the radioactivity in the plasma (dpm/ml) and lavage joint cavity (dpm/joint) 3 hours after $^3$H$_2$O injection.

Estimating joint fluid volume (Table IV)

The average joint fluid volumes estimated using calcium concentration were 401 µl/joint and 680 µl/joint in normal and arthritic rabbits, respectively, with a statistically significant difference found between the two values (p < 0.05). The average joint fluid volumes estimated using $^3$H$_2$O radioactivity were 425 µl/joint and 761 µl/joint in normal and arthritic rabbits, respectively, with a statistically significant difference found between the two values (p < 0.05). In both normal and arthritic rabbits, higher values were obtained in comparison with the estimation based on calcium concentration, but the values were still considered statistically insignificant.

The correlation between joint fluid volumes estimated using calcium concentration and $^3$H$_2$O radioactivity is shown in Figure 1. The joint fluid volumes of 12 joints (normal: 6 joints of 3 rabbits, arthritic: 6 joints of 3 rabbits) were determined by measuring the calcium concentration and $^3$H$_2$O radioactivity. As shown in the figure, there was a statistically significant relationship between the values obtained by these two methods, with a linear correlation coefficient (r value) of 0.985 (p < 0.01).

Table IV. Joint fluid volumes estimated by measuring calcium concentrations and radioactivity of $^3$H$_2$O.

<table>
<thead>
<tr>
<th></th>
<th>Ca concentration in the plasma (µg/ml)</th>
<th>Total Ca amount in the joint cavity lavage (µg/joint)</th>
<th>Joint fluid volume (µl/joint)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal rabbit</td>
<td>132 ± 3.15</td>
<td>52.7 ± 2.37</td>
<td>401 ± 23.4</td>
</tr>
<tr>
<td>Arthritic rabbit</td>
<td>126 ± 4.74</td>
<td>85.4 ± 8.42</td>
<td>680 ± 72.7*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Radioactivity in the plasma (10$^3$ dpm/ml)</th>
<th>Total radioactivity in the joint fluid lavage (10$^3$ dpm/joint)</th>
<th>Joint fluid volume (µl/joint)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal rabbit</td>
<td>193 ± 3.23</td>
<td>82.0 ± 8.29</td>
<td>425 ± 42.4</td>
</tr>
<tr>
<td>Arthritic rabbit</td>
<td>185 ± 1.21</td>
<td>140 ± 11.8</td>
<td>761 ± 63.1*</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.D. of 6 rabbits and 12 joints. *p < 0.05 versus joint fluid volume of the normal rabbit by Student’s t-test.
Discussion

In order to develop a new, simple, and more efficient method of estimating the joint fluid volume in experimental animals, the calcium concentration, which is maintained at a constant level in body fluid, was evaluated. As shown in Table II, there was no statistical significance in both calcium concentrations from the plasma and original joint fluid between normal and arthritic rabbits. Calcium concentration in the original joint fluid was only approximately 5% lower than in the plasma. These results indicate that the calcium concentration in plasma was the same as that in the original joint fluid in both normal and papain-induced arthritic rabbits.

Using the calcium concentration, the joint fluid volumes were estimated at 401 µl/joint and 680 µl/joint in normal and arthritic rabbits, respectively (Table III). On the other hand, the joint fluid volumes estimated using 1H2O were 425 µl/joint and 761 µl/joint in normal and arthritic rabbits, respectively (Table IV). Thus, there was no statistical significance between the joint fluid volumes obtained by these two methods. A statistically significant positive correlation was observed between the values from these two methods (r = 0.985, Fig. 1), indicating that the joint fluid volume estimated using calcium concentrations in the plasma and joint cavity lavage was appropriate.

However, the measured values in this study were approximately 3 times as high as the values (133µl / joint) reported by Delecrin et al. (6). Delecrin et al. determined the joint fluid volume by weighing a piece of cotton which had been inserted into the joint cavity to absorb fluid. Using their method, it is difficult to completely collect joint fluid. On the other hand, even the joint fluid absorbed in loose tissues such as synovium or adipose can be recovered by our method. Consequently, the joint fluid volume estimated according to our method was higher than that estimated using the Delecrin et al. method. The method developed in this study has one essential problem. That is, this method is constructed on the premise that the calcium concentration is maintained at a constant level in body fluid. This method therefore is limited to evaluating the efficacy of some anti-inflammatory medicines containing calcium as a counter ion due to influence its level in the plasma or joint fluid. In this case, it might be necessary to use this method for evaluation of the medicine after increased calcium concentration recovers to the original level. However, this simple and efficient method, which only involves the measurement of calcium concentrations colorimetrically in collected joint cavity lavage and plasma, is very useful when the joint fluid volume is determined in experimental arthritis disease models with a steady calcium concentration.

The joint fluid volume in arthritic rabbits was more than 1.5 times that in normal rabbits (Table IV). These results suggest that joint fluid volume can be used as a marker for experimental arthritis models. This method shows great promise in the evaluation of therapeutic medicine for arthritis or for hydroarthrosis research.

References