

**Structure and Size-selective Permeability of the Synovial Membrane of the
Temporomandibular Joint of the Mouse Measured by MR Imaging at 7T**

(7 T MRI を用いたマウス顎関節構造と滑膜における分子量選択的な透過性の解析)

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Abstract

Purpose: We analyzed the anatomical structure of the temporomandibular joint (TMJ) and molecular weight dependency of synovial membrane permeability in mice using 7-tesla magnetic resonance (MR) imaging.

Methods: We obtained 3-dimensional (3D) T_1 -weighted gradient echo (3D- T_{1w}) and 3D T_2 -weighted rapid acquisition with relaxation enhancement (3D- T_{2w} RARE) MR images of the TMJ of male C57BL6 mice with voxel resolution of 65 μm . Two-dimensional (2D) T_{1w} images were measured every 45 seconds before and after bolus intravenous (IV) injection of contrast reagents: gadolinium diethylenetriamine pentaacetic acid (Gd-DTPA; 0.5 kDa); oligomer-based contrast agent (CH3-DTPA-Gd; 2.1 kDa); gadolinium-labeled polylysine (Gd-polylysine; 10 kDa); and gadolinium-labeled albumin (Gd-albumin; 74 kDa).

Results: T_{1w} images depicted the temporal bone and mandibular condyle as regions with lower signal intensity and the disc as a region of intermediate intensity. In the Gd-DTPA-enhanced T_{1w} and T_{2w} images, the articular disc could be identified as a region with lower signal intensity than that of the upper and lower joint cavities. After IV injection of Gd-DTPA or CH3-DTPA-Gd, the signal intensity of the joint cavities increased within 10 minutes, but this increase was not shown with Gd-polylysine and Gd-albumin.

Conclusion: The structural findings obtained by MR imaging agreed with those obtained by hematoxylin-eosin staining under light microscopy. Contrast-enhanced MR imaging suggested that smaller (< 2.1 kDa) but not larger (> 10 kDa) molecules can permeate the synovial membrane. Our results suggest the utility of MR imaging for analyzing the structure of the TMJ as well as permeability of the synovial membrane.

Introduction

Inflammatory changes in the synovial membrane, cartilage, and disc characterize arthritis of the temporomandibular joint (TMJ),(1-4) and such structural changes as degradation or displacement of the disc are normally used to classify the stages of arthritis.(5-9) Inflammation is also known to precede the development of significant structural change,(10) and joint effusion is considered an early sign of arthritis.(10) In arthritis of the TMJ in humans and rabbits, increased concentrations of serum proteins in the synovial fluid suggest increased permeability of the vessels for these proteins.(11-13) Such an increase in vessel permeability in the synovial membrane may cause joint effusion.(10) Concentrations of Na^+ , K^+ , urea, and creatinine are almost the same in the synovial fluid and serum, whereas the concentration of serum proteins is much smaller.(14) Therefore, smaller molecules are highly permeable and permeability depends on molecular size for a molecule with a higher molecular weight. Thus, we measured the permeability of the intact synovial membrane in a nonarthritic state. Because gadolinium diethylenetriamine pentaacetic acid (Gd-DTPA) is rapidly enhanced in the joint cavity of the TMJ,(5,15) Gd-DTPA (0.5 kDa) must be able to permeate the synovial membrane. We also selected 3 contrast reagents—an oligomer-based contrast reagent (CH3-DTPA-Gd; 2.1 kDa), gadolinium-labeled polylysine (Gd-polylysine; 10 kDa), and gadolinium-labeled albumin (Gd-albumin; 74 kDa)—and tried to measure the molecular weight dependency of the permeability of the synovial membrane.

In this study, we attempted to visualize the TMJ in mice using 7-tesla MR microimaging with a small surface coil designed to fit the small TMJ of the mouse and to archive 65 μm resolution within one hour of image acquisition. We then evaluated the quality of the MR images and compared them with images obtained by hematoxylin-eosin staining. Finally, we applied 4 kinds of contrast reagents intravenously (IV), estimated the time constant of the inflow of the contrast reagent into the synovial

fluid in the TMJ based on the increase in the rate of longitudinal relaxation, and tried to detect the size-selective permeability of the synovial membrane.

Materials and Methods

Magnetic resonance microimaging of the mouse temporomandibular joint

The Animal Research Councils of Dokkyo Medical University approved all animal experiments. The related judgment reference numbers at Dokkyo Medical University are 726 and 769. We anesthetized male C57BL6 mice 5 to 36 weeks old with pentobarbital (50 mg/kg, i.p.) and inserted a venous catheter into the left femoral vein for infusion of the contrast reagents. The heads of the mice were placed in the lateral position on a custom-built plexiglass platform and fixed in place by adhesive tape. A ^1H radiofrequency (RF) surface coil (9 mm in diameter, Doty Scientific Inc., Columbia, SC, USA) was placed between the mouse eye and external auditory meatus to cover the left side of the TMJ with the sensitive area of the surface coil. The mice were anesthetized with 1% sevoflurane in a gas mixture of $\text{O}_2/\text{CO}_2/\text{N}_2\text{O}$ delivered through a face mask at a rate of 0.4 L/ min.

^1H MR images were obtained with a 7-T microimaging system (AVANCE III, Bruker BioSpin, Ettlingen, Germany) with ParaVision operating software (version 5.1, Bruker BioSpin). The parameters for 2-dimensional (2D) T_1 -weighted gradient echo imaging (T_{1w}) were: 16.64×16.64 -mm field of view (FOV), 256×256 data matrix, 0.5-mm slice thickness, 150-ms repetition time (TR), 4-ms echo time (TE), and 45° flip angle. Fat-suppressed 3-dimensional (3D) T_{1w} was also conducted with voxel size of $65 \times 65 \times 65 \mu\text{m}$ and a combination of TR/TE/flip-angle of 100 ms/4 ms/ 22.5° . Three-dimensional T_2 -weighted rapid acquisition with relaxation enhancement (RARE) imaging (3D- T_{2w}) was also conducted with voxel size of $65 \times 65 \times 65 \mu\text{m}$ and a combination of TR/TE/RARE-factor of 2000 ms/50 ms/16. A sinc-shaped pulse (duration 2 ms, bandwidth 2.5 kHz)

was used for excitation. The RF power was adjusted using a sagittal slice in the depth of the TMJ with one-mm thickness.

In accordance with slice setting for the rat TMJ,(15) we defined the coronal and sagittal planes of the MR imaging as follows. 1) We obtained a scout image showing the coronal direction (Fig. 1A) and defined the dorsal-ventral (D-V) direction using the dorsal sagittal sinus and longitudinal fissure of the cerebrum. 2) We then sliced the brain vertical to the D-V direction (Fig. 1B) and confirmed the longitudinal direction using the longitudinal fissure of the cerebrum. The left-right (L-R) direction was defined perpendicular to the longitudinal direction. 3) Finally, we sliced the midline of the brain parallel to the D-V direction and perpendicular to the L-R direction (Fig. 1C). The body of the basisphenoid bone beneath the brain base represented the head-foot (H-F) direction. The horizontal plane was defined as being parallel to the H-F direction and perpendicular to the D-V direction. The coronal plane was defined as a slice vertical to the horizontal plane and vertical to the longitudinal fissure of the cerebrum. The sagittal plane was defined as a slice vertical to the horizontal plane and parallel to the longitudinal fissure of the cerebrum.

Dynamic study of contrast-enhanced MR imaging of the mouse temporomandibular joint

We employed 4 kinds of contrast reagents for the dynamic study—Gd-DTPA (0.5 kDa) (Magnevist, Schering, Berlin, Germany), CH3-DTPA-Gd (2.1 kDa) (NMS-60, Nihon Medi-Physics, Chiba, Japan), Gd-polylysine (Gadopolylysine, 10 kDa, BioPAL, Worcester, MA, USA) and Gd-albumin (Galbium, 74 kDa, BioPAL, Worcester, MA, USA). Typical 2D-T_{1w} imaging parameters for dynamic study were: 5 coronal slices with voxel resolution, 65 × 65 μm; slice thickness, 0.5 mm with 0.7-mm separation; a combination of TR/TE/flip angle of 100 ms/4 ms/22.5°; and image acquisition, every 45 seconds. Four sets of images were obtained as a control group; a contrast reagent was injected within one minute via

the venous catheter with a syringe pump (TE-210, Terumo, Tokyo, Japan); and 15 sets of images were obtained every 45 seconds. Doses of reagents were Gd-DTPA, 200 nmol Gd/g body weight; CH3-DTPA-Gd, 200 nmol Gd/g body weight; Gd-polylysine, 127 nmol Gd/g body weight; and Gd-albumin, 7 to 10 nmol Gd/g body weight. The Gd-DTPA dose was a “double dose” used for the delayed Gd(DTPA)²-enhanced MR imaging.(16) Because the relaxivity of CH3-DTPA-Gd is similar to that of Gd-DTPA (personal communication from Nihon Medi-Physics), we set the dose of CH3-DTPA-Gd the same as that for the Gd-DTPA. The doses of Gd-polylysine and Gd-albumin were determined by preliminary experiments on the enhancement of vessels, and the results were similar to the doses recommended by BioPAL. To prevent differences in the loading volume, we adjusted the concentrations of the contrast reagents to the same volume as that of the injection (2 μ L/g body weight). For example, the concentration of Gd-DTPA in the injected solution was 100 mM. When 100 mM of Gd-DTPA is injected at a rate of 2 μ L/min/g body weight, the infused Gd-DTPA is instantaneously diluted by blood because the cardiac output of the mouse heart is 500 μ L/g body weight.(17) Indeed, mouse systemic blood could circulate 7 times per minute. Therefore, after one minute of injection, the plasma concentration of Gd-DTPA reached an almost constant level, which allowed us to treat the increase of Gd-DTPA concentration stepwise.

Two oral and maxillofacial surgeons with detailed knowledge of the temporomandibular joint in rats and humans and a physiologist with 34 years of experience in the field of the head and neck of mice and rats evaluated the MR and histological images. The mean image intensity of a small region of interest (ROI) in the lower joint cavity, temporal brain, temporal muscle, and transverse facial artery and vein were measured (Fig. 4), after which histological sections were prepared. Compared with the histological images, the MR images of the articular disc, mandibular condyle with cartilage, temporal bone, and upper and lower joint cavities were determined retrospectively. After the experiments, the

mice were euthanized with an overdose of pentobarbital (500 mg/kg i.p.).

Histological tissue preparation

The mice TMJs were dissected, fixed in 10% formalin neutral buffer solution, demineralized in 5% ethylenediaminetetraacetic acid (EDTA), and embedded in paraffin. Paraffin sections were prepared using a slice thickness of 8 μm and stained by H-E staining.

Statistics

Quantitative results were presented as means \pm standard error of the means. We performed statistical and regression analyses using Excel statistics routines (Microsoft, Redmond, WA, USA).

Results

Magnetic resonance microimaging of the mouse temporomandibular joint

Figure 1D and E shows a typical pair of T_{1w} images and their slice positions with pixel resolution of 65 μm and slice thickness of 0.5 mm. The images depict the temporal bone (a) and mandibular condyle (b) as regions of lower signal intensity, the articular disc (c) with intermediate signal, and the upper and lower joint cavities with higher intensity above the articular disc, but it was difficult to discriminate the upper and lower joint cavities from the cartilage.

To obtain the detailed structure of the TMJ, we measured the 3D- T_{1w} image and T_{2w} images with a voxel resolution of 65 μm , and were assigned in accordance with the H-E staining findings of the TMJ (Fig. 2). Sagittal images are routinely used for histology and clinical MR imaging of the TMJ. In comparison with a sagittal section of the TMJ stained with H-E (Fig. 2A), the upper temporal bone, lower mandible, and articular disc could be discriminated in the T_{1w} and T_{2w} images (Fig. 2B, C). The

temporal bone and mandibular condyle were depicted as regions of lower signal intensity and the disc, of intermediate intensity, with a biconcave shape; the posterior and anterior bands of the disc were thick; and the intermediate band of the disc was thin (Fig. 2B). A coronal direction image (Fig. 2D, E) presented a cross-section of the thick part of the disc. From the internal to the external side, the thickness of the disc was almost constant. This was also true for the central thin and the anterior thick parts. The articular cartilage of the mandibular condyle and the temporal bone were depicted as regions of highest intensity in the T_{1w} images (Fig. 2B, E). The upper and lower joint cavities were depicted as the regions of higher signal intensity above and below the articular disc, but it was difficult to distinguish the edge of the articular cartilage in the T_{2w} images (Fig. 2C, F). Figure 3 shows plain and Gd-DTPA-enhanced T_{1w} images. After an IV injection of Gd-DTPA, image intensity around the disc increased, and the position and shape of the disc were clearer than on images obtained without Gd-DTPA. Therefore, the regions of higher signal intensity around the disc in the T_{2w} images (Fig. 2C, F) and Gd-DTPA-enhanced images (Fig. 3C, D) represent synovial fluid in the upper and lower joint cavities.

Estimation of inflow of contrast reagents into the synovial fluid

Figure 4 shows typical results. After IV injection of one of the contrast reagents, image intensities of the transverse facial artery and vein were increased, reaching plateau levels within 3 minutes, except for the case of Gd-polylysine. The image intensity of the brain remained unchanged with all of the contrast reagents. The image intensities of the TMJ and muscle were increased by Gd-DTPA and CH3-DTPA-Gd (Fig. 4D, E) but showed no change with Gd-polylysine and Gd-albumin (Fig. 4H, I), suggesting that both Gd-DTPA and CH3-DTPA-Gd passed through the capillaries in the synovial membrane into the synovial fluid.

To estimate the inflow of the contrast reagents into the synovial fluid, we employed a 2-compartment model to analyze signal intensities observed on T_{1w} images.(18) After its bolus IV injection, a contrast reagent mixes rapidly and should maintain a steady level for 12 minutes. The synovial membrane acts as a semipermeable membrane to control molecular traffic into and out of the joint space.(19) A smaller contrast reagent, such as Gd-DTPA, could pass through the synovial membrane and appear in the synovial fluid. The concentration of the shift reagent ($C(t)$) can be written as: $C(t) = c (\exp(-k_o t) - \exp(-k_i t))$ Eq. [1], in which k_o and k_i are inflow and outflow of the contrast reagent, and c is a constant. In the case of slow outflow of the shift reagent, the equation can be simplified: $C(t) = c (1 - \exp(-k_i t))$ Eq. [2].

The longitudinal relaxation rate of the synovial fluid, ($R_1(t)$), is increased as: $R_1(t) = R_0 + K \cdot C(t)$ Eq. [3], where R_0 is the intrinsic longitudinal relaxation rate of the synovial fluid and K , the relaxivity value of the contrast reagent. The initial increase in the image intensity of the T_{1w} image ($\Delta M(t)$) with a short echo-time could be written as: $\Delta M(t) = M_O [\exp(-TR \cdot R_1(t)) - \exp(-TR \cdot R_0)] \approx K \cdot C(t) = K \cdot c (1 - \exp(-k_i t))$ Eq. [4], where M_O is the equilibrium image intensity, and TR is the repetition time of the excitation pulse. The first term of Eq. 4 corresponds to the plateau level at the end of the dynamic experiment (ΔM_{PL}). The mean values of the increase of the last 3 sets of images (13th to 15th) were used as ΔM_{PL} . Therefore, k_i can be obtained as the slope of $\log_e(\Delta M_{PL} - \Delta M(t))$ vs t , as shown in Figure 4F and G. The average k_i values for Gd-DTPA, 0.76 ± 0.22 ($n = 4$), and for CH3-DTPA-Gd, $0.63 \pm 0.09 \text{ min}^{-1}$ ($n = 3$), did not differ significantly ($P > 0.05$).

Discussion

The first objective of this study was to visualize components of the mouse TMJ. In a previous study of the TMJ of rats, we demonstrated visualization of the articular disc, articular cartilages, and

upper and lower joint cavities using 4.7-T MR imaging.(15) Because the TMJ of the mouse is much smaller than that of the rat, we employed a higher field (7 T) and smaller surface coil in this study to improve the spatial resolution from 100 μm to 65 μm . As shown in Figures 2 and 3, we visualized the articular disc, temporal bone, and mandibular condyle. Though we could not clearly distinguish between cartilage and synovial fluid on the T_2 -weighted images, we could visualize the synovial fluid in the joint cavity using contrast-enhanced MR images. Therefore, in addition to investigating the rat TMJ, we could study the mouse TMJ using high field MR images.

The second objective of this study was to analyze the molecular weight dependency of the permeability of the synovial membrane. To minimize the effects of the intravenous infusion of the contrast reagents, we minimized the amount and volume of contrast reagents. The volume of the contrast reagents (2 $\mu\text{L/g}$ body weight) were 3% of the total blood volume (70 $\mu\text{L/g}$ body weight).(17) The final plasma concentration of Gd-DTPA and CH₃-DTPA-Gd were estimated as 3 mM, and the plasma osmolality might increase by 6 mOsm/kg H₂O, which is also within normal range of changes in rodents' plasma osmolality (20 mOsm/kg H₂O).(20) The dose of Gd-albumin, one nmol albumin/g body weight, is only 1.5% of the total plasma proteins. Therefore, the intravenous injection of the contrast reagents should not affect the permeability of the synovial membrane. As shown in Figure 4, this method could detect a rapid exchange with a short time constant, in the order of 5 minutes. Equation 4 suggests that slower exchange could be detected if the concentration of the contrast reagent is increased. However, as discussed above, increases in the dose of contrast reagents will break down the physiological condition of mice. Thus, this method is not suitable for slower exchanges in the order of hours.

We used the contrast reagent Gd-DTPA to estimate the volume of extracellular water.(21,22) The injection of contrast reagents did not change image intensity of the brain. It is reasonable that none of

the contrast reagents could pass the blood-brain barrier because the blood-brain barrier of the brain capillaries could not pass the Gd-DTPA, and the partial volume effect of vessels could be minimum because the volume of plasma is around 1% of the total water in the rat brain.(20) Gd-DTPA and CH3-DTPA-Gd increased the image intensities of the muscles and TMJ (Fig. 4D, E), and the increase (ΔM_{PL}) was smaller in the muscles than the TMJ. Because the volume fraction of the extracellular fluid in the skeletal muscle of rats is around 16%,(20) it is likely that the volume fraction of the synovial fluid is dominant in ROIs located on the TMJ. Gd-polylysine and Gd-albumin did not change image intensities (Fig. 4H, I). These results suggest that smaller molecules (< 2.1 kDa) can permeate the synovial membrane, but larger molecules (> 10 kDa) could not pass within 10 minutes.

It is well known that small molecules, such as electrolytes, urea, and creatinine, easily permeate capillaries in the synovial membrane and are in equilibrium with small molecules of plasma.(14) Serum proteins are also found in the synovial fluid, and ratio of the concentration in the synovial fluid to that in the serum (SF/S) decreases progressively as molecular size increases.(14,23) The reported value of the SF/S ratio (0.48) for the major limb joints of rabbits(24) seems controversial, judging by our results, but this discrepancy is probably due to the difference in the time constant of the exchange. The SF/S ratio reflects a slow exchange with a long time constant between the serum and synovial fluid, whereas the inflow rate of contrast reagents reflects a rapid exchange with a short time constant, in the order of 5 minutes.

In conclusion, we could visualize the structure of the mouse TMJ by 7-T MR imaging, and the structural findings obtained by MR imaging agreed with those obtained by hematoxylin-eosin staining under light microscopy. Contrast-enhanced MR imaging suggested that smaller molecules (< 2.1 kDa) can permeate the synovial membrane, but larger molecules (> 10 kDa) cannot. The smaller contrast reagents, Gd-DTPA and CH3-DTPA-Gd, might be useful in evaluating the circulation and volume of

joint effusion. Larger contrast reagents, such as polylysine-Gd (10 kDa) and Gd-albumin, might be useful in evaluating changes in the vascular permeability of the synovial membrane. Our study results suggest MR imaging as a useful technique for analyzing the structure of the TMJ and permeability of the synovial membrane. To confirm these speculations, we plan to investigate changes in the molecular size-dependent permeability of contrast reagents in arthritis of the TMJ induced by anti-type II collagen antibody (25) in the future.

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Fig. 1. Coronal and sagittal T_1 -weighted magnetic resonance (MR) images of the mouse temporomandibular joint. Scout images of the (A) dorsal-ventral (D-V) direction, (B) left-right (L-R) direction, and (C) head-foot (H-F) direction. (D) Coronal plane image. The coronal plane was defined as a slice vertical to the base of the brain and vertical to the midline. The dashed line in the inset shows slice position. (E) Sagittal plane image. The sagittal plane was defined as a slice vertical to the base of the brain and parallel to the midline. The dashed line in the inset shows slice position. Labeled features: (a) temporal bone, (b) mandibular condyle, and (c) articular disc.

Fig. 2. Hematoxylin-eosin (H-E) staining and T_1 -weighted (T_{1w}) and T_2w magnetic resonance (MR) imaging of the sagittal and coronal sections of the mouse temporomandibular joint. (A) Sagittal plane image of hematoxylin-eosin (H-E) staining ($\times 40$). Scale bar, one mm. (B) Sagittal slice shown in a 3-dimensional (3D) T_{1w} gradient echo image, and (C) sagittal slice shown in a 3D T_{2w} rapid acquisition with relaxation enhancement (RARE) image. (D) Coronal plane image of H-E staining ($\times 40$). (E) Coronal slice shown in a 3D- T_{1w} gradient echo image and (F) coronal slice shown in a 3D- T_{2w} RARE image. Slice positions, shown by the yellow lines in the insets, were selected to match the H-E stained sagittal or coronal section. Labeled features: (a) temporal bone, (b) mandibular condyle, and (c) articular disc.

Fig. 3. Gadolinium diethylenetriamine pentaacetic acid (Gd-DTPA) enhancement of T_1 -weighted (T_{1w}) magnetic resonance (MR) imaging of the mouse temporomandibular joint. (A) Coronal slice shown in a plain T_{1w} gradient echo image and (B) the same coronal slice image after intravenous (IV) injection of Gd-DTPA. (C) Sagittal slice shown in a plain T_{1w} gradient echo image and (D) the same sagittal slice image after IV injection of Gd-DTPA. Signal intensity of joint cavities was depicted as

high and that of the articular disc as intermediate by the Gd-DTPA. Labeled features: (a) temporal bone, (b) mandibular condyle, and (c) articular disc.

Fig. 4. Dynamic study of contrast-enhanced magnetic resonance (MR) imaging of the temporomandibular joint and adjacent tissues of the mouse. (A) Coronal slice shown in a plain T_1 -weighted (T_{1w}) gradient echo image and (B) the same coronal slice image after intravenous (IV) injection of CH3 gadolinium diethylenetriamine pentaacetic acid (DTPA-Gd) at 0 minutes. (C) Coronal slice image after IV injection of Gd-albumin in different mice. The 4 boxes indicate the position and size of the regions of interest (ROIs). (D) and (E) Changes in image intensity of 4 ROIs after IV injection of Gd-DTPA and CH3-DTPA-Gd, respectively. (F) and (G) Fitting data for ROI of the temporomandibular joint after IV injection of Gd-DTPA and CH3-DTPA-Gd, respectively. The bold lines show the results of fitting to $\log_e(\Delta M_{PL} - \Delta M(t)) = -k_i t + B$, where $\Delta M(t)$ is the increase of image intensity, ΔM_{PL} is the mean value of the increase of the last 3 sets of images, and B is a constant. The inflow rate (k_i) of Gd-DTPA is 0.45 min^{-1} ($r = 0.99$), and that of CH3-DTPA-Gd is 0.71 min^{-1} ($r = 0.98$). (H) and (I) Changes in the image intensity of the 4 ROIs after IV injection of Gd-polylysine (10 kDa) and Gd-albumin, respectively, at 0 min.