Intracerebral drug delivery treatment using a regularly-structured biodegradable gel for slow-release of gadolinium-containing nano-micelles in a glioblastoma model

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Introduction Intracerebral drug delivery treatments, including convection-enhanced delivery, are a promising means to deliver therapeutic drugs beyond the blood-brain barrier (BBB) to treat brain tumors.[1,2] The drugs are directly and continuously injected into the brain tumor using a syringe or catheter (Fig. 1a). To obtain good therapeutic efficacy with minimal side-effects, it is important to control and evaluate the distribution of the injected drugs with minimal leakages out of the tumor, especially to the CSF (Fig. 1b). Previous research has been performed using a slow-infusion system under MRI monitoring to avoid fatal leakages from the tumor region.[3-6] In our research, we have developed a strategy to combine MR-visible micelles[7] and regularly-structured biodegradable gels to control drug release, to deliver the drug to the whole tumor with minimal leakage (Fig. 1c), and also to monitor the drug distribution using MRI. We present here studies evaluating *in vivo* gel stability and *in vitro* release of the Gd-containing micelles (Gd-micelles) from the gel, as well as serial MRI monitoring after *in vivo* gel injection into rat brain tumor.

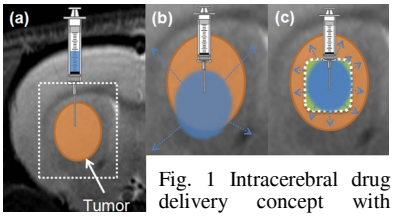

Fig. 1 Intracerebral drug delivery concept with MR-visible micelles and biodegradable gel. After the drug injection into the tumor area (a, orange), the micelles (b, blue) rapidly diffused around the tumor and may enter the CSF. When a gel is used, the micelle diffusion is confined to the tumor (c, green).

Materials and Methods Tetra-PEG gel, which has a homogeneous lattice-shaped structure, was produced by mixing phosphate buffered saline (PBS) with two polymer materials (tetra-amine-terminated PEG (polyethylene glycol) (TAPEG) and tetra-NHS (N-hydroxysuccinimide)-glutarate-teminated PEG (THPEG) (NOF Corporation, Japan)).[8] The rate of biodegradation depends on the polymer concentration. Gd-containing polymeric micelles (z-average hydrodynamic diameter: ~80 nm) were conjugated with Gd-DOTA and proteasome-inhibitor, MG-132. MR images were acquired on preclinical 20 cm bore (Bruker-Biospin, Germany) and 40 cm bore (Magnet: Kobelco and Jastec, Japan, Console: Bruker-Biospin) 7.0 Tesla MRI systems.

Gel stability *in vivo*: To evaluate the gel stability *in vivo*, gels with different polymer concentration were implanted at four subcutaneous regions of a BALB/c nude mouse (Japan SLC Inc.) and measured with T_2-weighted (T_2W) MRI for 2 weeks. MR images were acquired with the rapid acquisition with relaxation enhancement (RARE) sequence and the following parameters: TR/TE = 4,200 / 36 ms, FOV = 38.4 × 38.4 mm^2, slice thickness = 1.0 mm, Matrix = 256 × 256, RARE factor = 8, and NEX = 4.

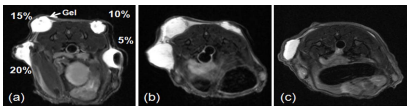

Fig. 2 Stability of gels with different polymer concentration at (a) 0 days, (b) 3 days and (c) 2 weeks.

Micelle release *in vitro*: To evaluate the release rate of the Gd-micelles from the gel, MR signal changes for gel containing Gd-micelles (0.5 mM) were compared with those for gel containing bare Gd-DTPA (0.5 mM, Magnevist, Bayer Yakuhin Ltd., Japan) up to 42 hours after gel preparation. A gel with 15% polymer concentration was used. The Gd-micelles or Gd-DTPA was mixed with Tetra-PEG gel in PCR tubes during gel production, and then PBS was poured on the gel. T_1-weighted (T_1W) MRI (TR/TE = 400/10.6 ms, FOV = 38.4 × 38.4 mm^2, slice thickness = 2.0 mm, Matrix = 256 × 256, and NEX = 4) and T_1-mapping were performed. Images were continuously acquired up until 14 hours after gel preparation, and then at 26 and 42 hours.

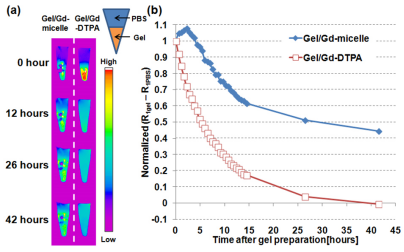

Fig. 3 Release of Gd-micelles and Gd-DTPA from the gel *in vitro*. (a) Typical T_1W images and (b) changes to (R_{1gel} - R_{1PBS}) normalized by the first image.

In vivo gel injection in the tumor: Male F344 rnu/rnu nude rats (CLEA Japan Inc.) were used. Human glioblastoma U87MG cells (3.0 × 10^5 cells /6 μl) were inoculated orthotropically. We developed a dual lumen catheter having a single outlet (inner diameter: 0.5 mm) that allows the two polymer solutions to mix inside the tip of the tube immediately prior to injection. The catheter was inserted into the tumor region using a stereotaxic instrument, and identical doses of the TAPEG with 0.25 mM Gd-micelles and THPEG solutions were injected at the same speed though the dual lumen catheter. The total dose was 20 μl (10 μl for each polymer). As a control, 0.25 mM Gd-micelles without gel were injected using the same procedure. MR images (T_1W and T_2W) were acquired using the RARE sequence before, immediately after, and at 1, 2 or 3 days after the administration. The parameters were as follows: T_1W image: TR/TE = 400/8.7 ms, FOV = 25.6 × 25.6 mm^2, Slice thickness = 1.0 mm, Matrix = 256 × 256, RARE factor = 2, and NEX = 16; and T_2W image: TR/TE = 4,200 / 36 ms, NEX = 4 and other parameters were the same as for the T_1W image.

Results and Discussion Figure 2 shows changes to the subcutaneously implanted gel signal over 2 weeks. The T_2W signal of the 5% and 10% polymer gels disappeared within 3 days (2a, b), while the signal from the 20% polymer gel remained for over 2 weeks (2c). Thus, a polymer concentration of 15% was used in subsequent experiments as it provides slow-release of the Gd-micelles from the gel over several days. In the *in vitro* experiment the signal from the gel with Gd-micelles was always higher than that of the PBS (Fig 3). On the other hand, the signal from gel with bare Gd-DTPA decreased rapidly and was similar to the PBS signal at 26 hours. These results indicate that the release of Gd-micelles from the gel continues slowly for several days. Figure 4 presents MR images before and after the injection of the Gd-micelles with or without gel. When gel with Gd-micelles was injected, the MR signal was locally enhanced near the injected area (orange arrows) without minimal leakage to the ventricle (yellow arrow). The T_1W signal enhancement slowly decreased (purple arrow) and was maintained at 2 or 3 days after injection even though some gel remained (blue arrow). This indicates that the Gd-micelles were slowly and continuously released from the gel in the tumor. In contrast, substantial leakage of Gd-micelles from the tumor to the ventricle was observed when no gel was used (green arrows). Moreover, the Gd-micelles remained outside the tumor including the ventricle for over 2 days (red arrows) without any therapeutic effect.

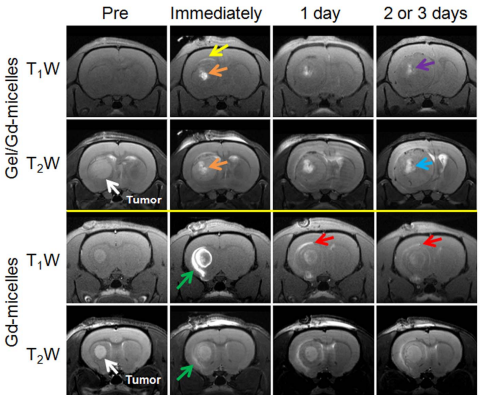

Fig. 4 Results after i*n vivo* injection of the gel with Gd-micelles and Gd-micelles only in a tumor region.

Conclusion: Our strategy using Gd-micelles with biodegradable Tetra-PEG gel allows slow and controlled release of the drug with MRI monitoring and has the potential to provide effective intracerebral drug delivery treatment with minimal side-effect.

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