Creation of Cisplatin-Adsorbing Regenerative-Medicine Gelatin Sponge and Its Cisplatin Release Pattern

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The purpose of this study was to clarify the adsorption of cisplatin on regenerative-medicine (RM) gelatin sponge, and to verify the relationship between the cisplatin release pattern of cisplatin-adsorbed RM gelatin sponge and the dissolving time of RM gelatin sponge. We tested various RM gelatin sponges, one with a molecular weight of 50000 Daltons (RM-50 gelatin sponge) that is 100% saline soluble at 24 h, RM-50-120 (heated at 120°C) that is 54.3% saline soluble at 24 h, and RM-50-140 (heated at 140°C) that is 15.8% saline soluble at 24 h. We investigated the production of cisplatin-adsorbed RM gelatin sponge and measured free cisplatin released from cisplatin-adsorbed RM gelatin sponge. There was no significant difference in the weight of adsorbed cisplatin among the RM-50, RM-50-120, and RM-50-140. The results mean that cisplatin adsorbs onto RM gelatin sponge irrespective of heating temperature. The average adsorbed weight of cisplatin per gram of RM gelatin sponge was 29.3 mg, which was approximately five times more than that previously reported for Gelpart (non-soluble gelatin sponge, clinically available). Cisplatin release in the RM-50 gelatin was the most rapid at only 1 h after incubation; it was released gradually and increasingly in the RM-50-120 gelatin, and released slowly in the RM-50-140 gelatin for 24 h incubation. Cisplatin-adsorbed RM gelatin sponge released cisplatin proportional to the dissolving time of RM gelatin sponge, indicating that the cisplatin release time can be controlled by heating for sterilization of RM gelatin sponge.

Key words regenerative-medicine (RM) gelatin; endotoxin; cisplatin; drug delivery system (DDS); trans-catheter arterial chemoembolization (TACE)

Gelatin has been extensively used by pharmaceutical manufacturers as a pharmaceutical excipient (stabilizer, additive, etc.) Furthermore, it is used as a material for biomaterials. For example, it is used in the biomaterial sheet to facilitate regeneration of dermis to cover a skin deficit.1,2) The endotoxin in gelatin is lipopolysaccharide (LPS), which is the same component as the outer envelope of Gram-negative bacteria, and is commonly isolated from Gram-negative bacteria. The collapse of the cell wall due to autolysis and denaturation would cause much more LPS to be released, damaging various types of cells.3–5) When LPS invades the body, it causes fever and can cause septic shock.6–8) Sterilization to 121°C is insufficient to eradicate endotoxic activity, this requires heating at 250°C for more than 60 min.9) It is preferable to use gelatin without bacteria or endotoxins when this biomaterial is to be introduced into the body. The gelatin that matches the standard for purified gelatin in Japanese Pharmacopoeia in this regard was developed in 2007, and is termed regenerative-medicine (RM) gelatin. RM gelatin was removed endotoxin using a ultra filtration membrane without heat sterilization at 250°C.10)

We created cisplatin-adsorbing RM gelatin sponge. The solubility of dried RM gelatin sponge can be controlled by heating for sterilization.15) Although transcatheter arterial chemoembolization (TACE) with insoluble gelatin sponge and anticancer drugs for hepatocellular carcinoma (HCC) is known to cause hepatic arterial damage,12,13) Kawai et al. reported that TACE with soluble gelatin sponge for HCC resulted in the same therapeutic effect as TACE with non-soluble gelatin sponge, while causing significantly less hepatic artery impairment.14) When anticancer agents are commonly used in a lipiodol emulsion in TACE of the hepatic artery for HCC, it is reported in the experimental study that anticancer agents was washed out rapidly from the liver to the hepatic vein.19) We hypothesized that the RM gelatin sponge itself may adsorb the anticancer drugs and also control the release, and could thus be used in the purpose for a chemo-carrying material.

The purpose of this study is to clarify the adsorption of cisplatin on RM gelatin sponge and to verify the relationship between the release pattern of cisplatin from cisplatin-adsorbed gelatin sponge and the dissolving time of RM gelatin sponge. In this study, we selected aseptic and least endotoxic RM gelatin, with consideration to its use in procedures such as TACE.

MATERIALS AND METHODS

RM Gelatin Sponge We used the least endotoxic gelatin sponge (RM-50: RM gelatin sponge, average molecular weight approximately 50000 Dalton; Jellice Co., Sendai, Japan) originating from acid-treated pig skin (Fig. 1). We have found that the solubility of the gelatin is different depending on sterilization temperature. Four kinds of RM-50 gelatin sponge were created by controlling the temperature of heating for sterilization. We heated RM-50 gelatin sponge at 120, 130, 140, and 150°C for 24 h, to produce RM-50-120, RM-50-130, RM-140, and RM-50-150, respectively.

Procedure for Testing the Solubility of RM Gelatin Sponge in Saline The RM gelatin sponge was cut into approximately 3 mm cubes with scissors. Half gram of the cubes was inserted into a test tube with 10 mL saline and placed in an incubator at 37°C with vibration. Twenty four hours later, the supernatant was removed and the gelatin concentration was measured. It was regarded as 100% solubility when 0.5 g of the gelatin sponge has completely dissolved (5% gelatin

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**Histological Examination** with hematoxylin and eosin stain. The layers surrounding the RM gelatin sponge were removed for 30 d after implantation. Samples of the soft tissue and muscle implanted RM gelatin sponge pieces at 1 d after, 7 d after, and insertion and on the day after re-incision.

**Penicillin Administration** A single dose of penicillin (0.5 mL/kg) was administered on the day of insertion. A venous infusion of penicillin (Meiji Seika Co., Tokyo, Japan) was started after the incision was made. The wound was sutured under intraperitoneal administration of general anesthesia with intraperitoneal administration of pentobarbital (Somnopentyl, Kyoritsu Co., Tokyo, Japan). After iodine sterilization, a skin incision was made in the scapular region using a razor and sufficient space was created to insert a 10 mm cube of RM gelatin sponge between the left scapular region. The weight of dried RM gelatin sponge for implantation was 0.3 ± 0.02 g for RM-50, 0.3 ± 0.03 g for RM-50-120, and 0.3 ± 0.03 g for RM-50-140. The weight of dried RM gelatin sponge was 0.3 ± 0.02 g (mean ± S.D.) for RM-50, 0.3 ± 0.03 g for RM-50-120, and 0.3 ± 0.03 g for RM-50-140. After insertion, the skin was sutured under intravenous infusion of penicillin (Meiji Seika Co., Tokyo, Japan) and the rats were returned to their cages. Intramuscular injection of penicillin (0.5 mL/kg) was administered on the day of insertion and on the day after re-incision.

Re-incision was performed to observe and weigh the implanted RM gelatin sponge pieces at 1 d after, 7 d after, and 30 d after implantation. Samples of the soft tissue and muscle layers surrounding the RM gelatin sponge were removed for histological examination with hematoxylin and eosin stain.

We obtained approval from our Institutional Committee on Animal Welfare (SCAW) classification. Accordingly, care of the health status of the rats during and after the procedure was placed under the direction of a veterinarian.

**Method for Creation of Cisplatin-Adsorbed RM Gelatin Sponge** One tenth of a gram of the 3 mm cubes (RM-50, RM-50-120, and RM-50-140) was inserted into a test tube with 10 mL saline at room temperature. We dissolved 20 mg of cisplatin per 0.1 g of RM gelatin sponge in saline warmed at 50°C; this solution was added to the saline containing the RM gelatin sponge. Cisplatin was adsorbed on the RM gelatin sponge cubes by mixing for several minutes. Cisplatin solution and cisplatin-adsorbed-gelatin sponge particles were placed in a dialysis tube (sequestrated molecular weight 6000–8000 Dalton). The tube was placed in a container containing saline to eliminate non-adsorbed cisplatin, and dialysis was performed at 4–10°C. Saline was sampled from outside the dialysis tube and cisplatin concentration was measured; thereafter, all saline was replaced at 1, 3, 6, 21, and 46 h. Cisplatin measurement was performed at 6, 21, and 46 h by quantitative platinum analysis using the inductively coupled plasma method (ICP). Dialysis was discontinued when cisplatin was not detected in the saline outside the dialysis tube, indicating successful production of cisplatin-absorbed RM gelatin sponge cubes.

**Measurement of Free Cisplatin out of Cisplatin-Adsorbed RM Gelatin Sponge** To measure non-adsorbed cisplatin released from the cisplatin-adsorbed RM gelatin sponge, 0.1 g of cisplatin-adsorbed RM gelatin sponge cubes (RM-50, RM-50-120, and RM-50-140) was placed in a test tube with 10 mL saline, and then incubated at 37°C with vibration. The supernatant was immediately removed for sampling at 1 and 24 h, and platinum was measured by ICP analysis. At 24 h, all RM gelatin sponge cubes were forcefully dissolved by the addition of collagenase type I (protease, Wako Pharmaceutical Co., Tokyo, Japan), resulting in the release of all cisplatin. We calculated the released platinum per 1.0 g of RM gelatin sponge. The water content of the RM-50 is very low because it is a freeze-dried product. In addition, the influence is less than the RM-50-140 and RM-50-120, which is heat treated.

**RESULTS**

**Solubility of RM Gelatin Sponge: in Vitro Study** The solubility of the various RM gelatin sponges in saline are shown in Table 1. Solubility decreased with increased temperature for sterilization. Significant difference was found among the five RM gelatin sponges for solubility in saline. Because the most pronounced difference was found among RM-50 (solubility of 100%) and RM-50-140 (solubility of 15.8 ± 3.3%). These RM gelatin sponges were selected for use in the Solubility of RM gelatin sponge in vivo study and the Release of cisplatin from cisplatin-adsorbed RM gelatin sponge in vitro study, as follows.

**Solubility of RM Gelatin Sponge in Vivo Study** We performed macroscopic examination of the RM gelatin sponges implanted in the subcutaneous tissue (Fig. 2). RM-50 gelatin sponge began to dissolve immediately after implantation, and

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**Table 1. Solubility of RM Gelatin Sponge in Vitro Study**

| Heated temperature (°C) | Solubility (%) |
|-------------------------|----------------|
| RM-50                   | 0              | 100            |
| RM-50-120               | 120            | 54.3 ± 10.7    |
| RM-50-130               | 130            | 25.3 ± 5.6     |
| RM-50-140               | 140            | 15.8 ± 3.3     |
| RM-50-150               | 150            | 6.0 ± 2.2      |

All gelatin was heated for 24 h, except RM-50.
was completely dissolved within one day. Some RM-50-120 gelatin sponge was present the following day, but had completely dissolved at 7 d. RM-50-140 gelatin sponge was present the following day and at 7 d, and was partially dissolved at 30 d.

Microscopic examination of the tissue surrounding the RM gelatin sponge revealed no histological necrosis or degeneration, and no infiltration of inflammatory cells.

**Release of Cisplatin from Cisplatin-Adsorbed RM Gelatin Sponge**

Figure 3 shows the change in released platinum in the RM-50, RM-50-120, and RM-50-140 cubes immediately after, at 1 h, and at 24 h after addition of protease and incubation with vibration. The most cisplatin was released at 1 h after incubation in RM-50; it was released gradually and increasingly for 24 h in RM-50-120, and released slowly for 24 h in RM-50-140 (Fig. 3). All cisplatin was released from cisplatin-adsorbed RM gelatin sponge when protease was used. Total released cisplatin per g for RM-50, RM-50-120, and RM-50-140 gelatin sponge was 30.3±1.5 mg (mean±S.D.), 29.3±2.3 mg, and 28.3±3.2 mg, respectively.

**DISCUSSION**

Total released cisplatin weight per g for RM-50, RM-50-120, and RM-50-140 gelatin sponge was 30.3±1.5 mg (mean±S.D.), 29.3±2.3 mg, and 28.3±3.2 mg, respectively. Therefore, there was no significant difference in terms of the weight of adsorbed cisplatin among RM-50, RM-50-120, and RM-50-140. The weight of cisplatin adsorbed on RM gelatin sponge did not change by heating temperature for sterilization. The average adsorbed weight of cisplatin per g of RM gelatin sponge was 29.3±2.2 mg, which is approximately five times greater than that per g previously reported for Gelpart (non-soluble gelatin sponge, clinically available for TACE).16)

In this in vitro study, cisplatin was released instantly from cisplatin-adsorbed RM-50 gelatin sponge, released gradually from cisplatin-adsorbed RM-50-120 gelatin sponge, and released very slowly in RM-50-140 during the 24 h observation period.

On the other hand, gelatin sponge itself implanted in rat subcutaneous was found to disappear gradually. RM-50 gelatin sponge dissolved soon after implantation, RM-50-120 gela-
tin sponge was present the following day, but had completely dissolved at 7d. RM-50-140 gelatin sponge disappeared most slowly; approximately half had left after 30d.

In feasibility study in a swine model, cisplatin-adsorbed RM gelatin sponge functions as a cisplatin carrier.\(^7\) Cisplatin concentration in the artery is reduced quickly in intra-arterial administration group, it is cisplatin is released in the artery continuously was found in the combination of RM gelatin sponge and intra-arterial administration.

In this study, RM gelatin was shaped 3 or 10mm cube, but can be processed into particles of several hundred μm like Microspheres.\(^8\) Microspheres are an embolic agent that is commonly used in Europe. The microspheres are classified as a permanent embolic material made from polyvinyl alcohol. Therefore, the release mechanism of the anticancer agent adsorbed on the microspheres is not known.

In conclusion, microscopic examination of the tissue surrounding the implanted RM gelatin sponge revealed no histological necrosis or degeneration, and less infiltration of inflammatory cells. In addition, it is thought to be safe than Microsphere because it is not a permanent embolic material. So, we’re thinking of implanting RM gelatin as safe as an embolic material. The average adsorbed weight of cisplatin was 29.3±2.2mg of RM gelatin sponge, indicating that the cisplatin release can be controlled by heating for sterilization. To diminish undesirable side effects of chemical cross-linking of biodegradable materials, Ma et al. developed a thermal cross-linking method that involved esterification by dehydration under dry conditions.\(^9\) Then, the biodegradability of collagen is changed by thermal cross-linking. Changes in the solubility of RM gelatin may be due to the cross-linking occurred by heating.

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