A New Soluble Gelatin Sponge for Transcatheter Hepatic Arterial Embolization

Isao Takasaka · Nobuyuki Kawai · Morio Sato · Shinya Sahara · Hiroyuki Minamiguchi · Motoki Nakai · Akira Ikomu · Kouhei Nakata · Tetsuo Sonomura

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Abstract To prepare a soluble gelatin sponge (GS) and to explore the GS particles (GSPs) that inhibit development of collateral pathways when transcatheter hepatic arterial embolization is performed. The approval of the Institutional Committee on Research Animal Care of our institution was obtained. By means of 50 and 100 kDa of regenerative medicine–gelatin (RM-G), RM-G sponges were prepared by freeze-drying and heating to temperatures of 110–150°C for cross-linkage. The soluble times of RM-GSPs were measured in vitro. Eight swine for transcatheter hepatic arterial embolization were assigned into two groups: six received 135°C/50RM-GSPs, 125°C/100RM-GSPs, and 138°C/50RM-GSPs, with soluble time of 48 h or more in vitro; two swine received Gelpart GSPs (G-GSPs) with insoluble time of 14 days as a control. Transarterial chemoembolization was performed on two branches of the hepatic artery per swine. RM-GSPs heated at temperatures of 110–138°C were soluble. Mean soluble times of the RM-GSPs increased with higher temperature. Hepatic branches embolized with G-GSP remained occluded after 6 days, and development of collateral pathways was observed after 3 days. Hepatic branches embolized with 135°C/50RM-GSP and 125°C/100RM-GSP remained occluded for 4 h, and recanalization was observed after 1 day. Hepatic branches embolized with 138°C/50RM-GS remained occluded for 1 day, and recanalization was observed after 2 days with no development of collateral pathways. In RM-GSs with various soluble times that were prepared by modulating the heating temperature, 138°C/50RM-GSP was the soluble GSP with the longest occlusion time without inducing development of collateral pathways.

Keywords Interventional radiology · Transcatheter arterial chemoembolization · Embolic material · Hepatocellular carcinoma

Introduction

Gelatin sponge particles (GSP) have been used as an embolic material since transcatheter hepatic arterial embolization (THAE) was initially performed for hepatocellular carcinoma (HCC) in the late 1970s [1–4]. Repeated transarterial embolization that uses GSP is conducted for recurrent HCC, and is believed to increase the long-term survival rate of patients with HCC [5]. However, it commonly causes occlusion of the hepatic artery, facilitating the development of intrahepatic collateral pathways to recurrent HCC. Hepatic arterial collaterals after THAE have been identified and classified into two pathways: extrahepatic and intrahepatic. As extrahepatic collateral pathways, the inferior phrenic artery, lumbar artery, omental artery, dorsal pancreatic artery, left gastric artery, and internal mammary artery are recorded [5–10]. Extrahepatic collateral arteries often involve the blood supply of primary large HCC of 5 cm or more and develop with increased number of THAE sessions [11]. Selective catheterization of the extrahepatic collateral pathways is time-consuming and not always successful with intimal dissection a possible complication [8]. As intrahepatic collateral pathways, perivascular, interlobar, or intersegmental collaterals are often observed in follow-up angiography after THAE [9, 10].

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GSPs available for THAE are insoluble and are reported to be absorbed and/or embedded in the arterial wall, as is the biological response to a foreign body [12]. This response results in occlusion or stenosis with a narrow diameter even if the hepatic artery is recanalized [12]. The development of soluble GSP with an appropriate duration of occlusion would enable tumor ischemia while avoiding the occlusion and/or stenosis of the hepatic artery that may occur with embolization, and would inhibit the development of collateral pathways.

Gelatin, a fluid protein extracted from collagen, is used as a biomaterial and is denatured by the application of heat. The low-endotoxin gelatin that meets the standard for purified gelatin in the Japanese Pharmacopoeia was developed for regenerative medicine in 2007 [13]. The temperature of the dry heat used for sterilization determines the extent of cross-linkage [13]. We noticed that the solubility of dried gelatin sponge (GS) could be controlled depending on the extent of heat-induced cross-linkage. The objective of this study is to prepare a soluble GS and to explore the feasibility of GS particles that inhibit development of collateral pathways when THAE is performed.

Materials and Methods

We obtained the approval of the Institutional Committee on Research Animal Care of our institution before initiation of the study.

Preparation of the Gelatin with Heat Cross-linkage

We used low-endotoxin gelatin with molecular weights of 50 and 100 kDa regenerative medicine–gelatin (RM-G): 50RM-G and 100RM-G (Jellice, Sendai, Japan). This gelatin is preprocessed by ultrafiltration with a thin membrane to remove antigenicity and endotoxins. First, we prepared RM–gelatin sponge (RM-GS) with RM-G. Fluid gelatin was rapidly freeze-dried for 48 h at a temperature of −85°C to yield solid gelatin sponge (RM-GS). The RM-GS then underwent dry-heat sterilization for 24 h to cause heat-induced cross-linkage in the RM-GS. Heat-induced cross-linkage cannot be generated at a temperature less than 100°C [13]. The RM-GS was then heated at temperatures of 110, 120, 125, 130, 135, 138, and 150°C. After the heat-induced cross-linkage process, the RM-GSs were sliced into tiny 1-2 mm RM-GS particles (RM-GSPs).

Soluble Time of RM-GSP In Vitro

Twenty RM-GSP particles with a total weight of less than 100 mg were placed in a test tube to which 10 ml saline was added before being placed in a 37°C thermostat with vibration. The soluble time until complete lysis was measured for 2 weeks. Four measurements were performed for each of 10 different kinds of RM-GSPs. Gelpart GS particles of 1 mm in size (G-GSP; Nihonkayaku, Tokyo, Japan), which are legally approved for clinical use in Japan, were used as a study control.

Recanalization Time of the Hepatic Artery with Embolization with RM-GSP

We speculated that in the clinical setting, 2 days of ischemia would be sufficient to cause ischemic damage to HCC [14]. The results of the in vitro study indicated that the following three kinds of RM-GS with soluble times of 48 h or more in vitro are appropriate for in vivo study, with G-GS as a control (Table 1): RM-GS with 50 kDa, which was dry-heated at a temperature of 135°C (135°C/50RM-GS); RM-GS with 50 kDa, which was dry-heated at a temperature of 138°C (138°C/50RM-GS); and RM-GS with 100 kDa, which was dry-heated at 125°C (125°C/100RM-GS).

Eight healthy young female swine weighing from 52 to 60 kg (mean, 56.8 kg) were used in the study. The swine were assigned to the following groups: two swine received 135°C/50RM-GS, two received 138°C/50RM-GS, two received 125°C/100RM-GS, and two received G-GS as a control.

Each swine fasted for 12 h before the in vivo study. Preanesthesia was achieved with a combination of

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Soluble time of regenerative medicine gelatin sponge particles (RM-GSP) with heat induced cross-linkage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gelatin sponge particle</td>
<td>Molecular weight (kDa)</td>
</tr>
<tr>
<td>RM</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>50</td>
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<td></td>
<td>50</td>
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<tr>
<td></td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>100</td>
</tr>
<tr>
<td>Gelpart</td>
<td>a, b</td>
</tr>
</tbody>
</table>

Note: Data are means ± standard deviations
kDa kilodalton, NS not soluble for 14 days
a Used for in vivo study
b no information
c Information provided by the manufacturer (Nihon Kayaku, Tokyo, Japan)
30 mg/kg ketamine and 0.08 mg/kg atropine sulfate. The swine were intubated and connected to a small whole-body animal anesthesia apparatus. After intramuscular injection of 5 mg/kg xylazine and 5 mg/kg ketalar, general anesthesia was maintained with methoxyflurane inhalation. Cardiac and respiratory parameters were monitored, and a venous drip infusion of isotonic sodium chloride was continued throughout the procedure.

The femoral artery was punctured percutaneously. A 4F RC catheter (Medikit, Tokyo, Japan) was introduced into the celiac artery via a 4F sheath (Long sheath; Medikit). The common hepatic artery was catheterized with a 0.035-inch guide wire (Radifocus, Terumo, Tokyo, Japan), and angiography was performed to identify the proper hepatic artery and its hepatic branches. The medial and lateral branches of the left hepatic artery were catheterized with a 2.2F microcatheter (Tangent, Boston Scientific, Natick, MA) and a 0.014-inch micro-guide wire (Transend EX, Boston Scientific) for embolization. The GS was cut into 1-mm-sized particles that were soaked in contrast medium (Iopamidol 370; Bracco, Milano, Italy). The GSPs were slowly injected into the branches under fluoroscopic control. Two branches in each swine were embolized with the GSP until total occlusion was achieved. Oral antibiotics were administered from the following day for 3 days.

The recanalization of the two branches of the hepatic artery in each swine was explored for 6 days. Common hepatic arteriography was performed before, immediately after, and until recanalization: after 4 h, and at 1, 2, 3, 5, and 6 days. The arteriograms were interpreted by two independent interventional radiologists with more than 5 years of experience. Recanalization was defined as good blood flow runoff and clear visualization of the branch without the presence of filling defects corresponding to GSP.

Evaluation of Adverse Effects on the Liver

Peripheral blood was taken before embolization and again at 1, 3, and 5 days after embolization to assess changes in CRP (C reactive protein), white blood cells, AST (aspartate aminotransferase), total bilirubin, (GTP (glutamyl transpeptidase), and creatinine.

Histological Evaluation

The swine were sacrificed 6 days after embolization to evaluate the effect of embolization on their livers. Necropsies were performed and the livers removed. The livers were cut into sections of 10 mm thickness and fixed in a 7.5% neutral formaldehyde buffer. The liver specimen in each group was stained with hematoxylin and eosin for microscopic examination. The presence of GSP in the hepatic arteries and the extent of liver damage were assessed by macroscopic and microscopic examination. Namely, as liver damage, the presence or absence of coagulation necrosis and/or abscess was explored by macroscopic examination, and that of liver cell necrosis, degeneration, inflammatory cell infiltration to Glisson’s sheath, and arteritis and/or cholangitis was explored by microscopic examination. To determine the distribution of RM-GSPs in hepatic arteries of various diameters, we analyzed 60 microscope fields of view (20 microscopic fields x 3 times) at 100 x magnification for each of the three different RM-GSPs and G-GSPs as a control.

Statistical Analysis

Data are expressed as mean ± standard deviation. Student’s t-test was used to compare groups regarding the number of GSPs present in the hepatic arteries in 20 microscope fields of view, and Pearson’s χ² test was used to evaluate the distribution of GSPs with respect to the size of the hepatic arteries. A value of P < 0.05 was considered to be significant.

Results

Soluble Times of RM-GSPs In Vitro

The relations between the dry-heat temperatures of 50RM-GSP and 100RM-GSP, and the soluble time in saline are shown in Table 1. All RM-GSPs that were dry-heated at temperatures from 110 to 138°C were soluble. The mean soluble time of the RM-GSP increased with higher temperature. The mean soluble time was longer for 100RM-GSP than for 50RM-GSP at the same temperature (Fig. 1); specifically, heat-induced cross-linkage occurred at a lower temperature in 100RM-GSP than in 50RM-GSP. RM-GSP dry-heated at 150°C (150°C/100RM-GSP) and G-GSP were not soluble for 14 days.

Of the four kinds of RM-GSPs with soluble times in excess of 48 h, we chose three for the in vivo study: 135°C/50RM-GS, 138°C/50RM-GS, and 125°C/100RM-GS; G-GSP was used as a control (Table 1).

Occlusion Times of G-GSP and RM-GSPs In Vivo

Table 2 shows the occlusion times of the branches of the hepatic artery embolized with RM-GSP in follow-up hepatic angiography in vivo. Four branches of the hepatic artery embolized with G-GSP that was insoluble for 14 days in vitro remained occluded 6 days after embolization; the occlusion time of the branches in vivo was more than 6 days (Fig. 2).
Development of Intrahepatic Collateral Pathways

No development of collateral pathways was observed in follow-up hepatic arteriography from immediately after to 48 h after embolization with RM-GSP or G-GSP (Table 3). In contrast, collateral pathways were observed at follow-up hepatic arteriography 72 to 144 h after embolization with G-GSP; therefore, collateral pathways developed macroscopically under the condition of occluded hepatic arteries from at least 72 h after transarterial embolization.

Complications

Before the animals were killed, no adverse effects such as fever and/or death were observed. Blood tests, including white blood cells, CRP, AST, ALT, total bilirubin, and creatinine, were all within normal ranges.

Histological Study

Macroscopic and microscopic analysis of liver parenchyma showed no abnormal findings in any swine. Microscopic examination revealed that GSP existed in the hepatic arteries but not in the portal vein or hepatic sinusoids. GSPs were common in hepatic arteries with red thrombus in the swine embolized with G-GSP, whereas they were significantly less common in the swine embolized with 138°C/50RM-GS and 135°C/100RM-GS (P = 0.043), and were not observed in the swine embolized with 135°C/50RM-GS and 125°C/100RM-GS (Table 4). GSPs were mainly present in hepatic arteries with sizes of 100–500 μm and 500 μm or more in the swine embolized with G-GSP, and in those with sizes of 100–500 μm in the swine embolized with 138°C/50RM-GS. Significant difference in the distribution of GSPs was observed in terms of size of the hepatic arteries between the swine embolized with G-GSP and those embolized with 138°C/50RM-GS (P = 0.046) (Table 4).

Table 2. Recanalization of embolized hepatic artery after transcatheater arterial embolization with regenerative medicine gelatin sponge particles (RM-GSP) and Gelpart gelatin sponge particles (G-GSP) in follow-up hepatic arteriography

<table>
<thead>
<tr>
<th>Embolic materials</th>
<th>Number of embolized hepatic arteries</th>
<th>Number of recanalized hepatic arteries</th>
<th>Recanalization time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>RM-GSP 50 kDa/135°C</td>
<td>4</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>RM-GSP 100 kDa/125°C</td>
<td>4</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>RM-GSP 50 kDa/138°C</td>
<td>4</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>G-GSP</td>
<td>4</td>
<td></td>
<td>0</td>
</tr>
</tbody>
</table>

kDa: kilodalton
Fig. 2 Angiograms showing recanalization of the branches of the hepatic artery over time after embolization using Gelpart gelatin sponge particles insoluble for 14 days in vitro. Common hepatic arteriography was performed before (A), immediately after (B), and 6 days after embolization (C). The branches remain occluded (arrow) at 6 days after embolization, and development of intrahepatic collateral pathways (arrowhead) is observed.

Fig. 3 Angiograms showing recanalization of the hepatic artery over time after embolization using regenerative medicine gelatin sponge particles (RM-GSP, 50 kDa, 135°C heat cross-linkage) soluble at 57.6 h (2.4 days) in vitro. Common hepatic arteriography was performed before (A), 4 h after (B), and 1 day after embolization (C). The branches remain occluded at 4 h after embolization. Recanalization of the branches is observed after 1 day, with no development of collateral pathways.

Fig. 4 Angiograms showing recanalization of the hepatic artery over time after embolization using regenerative medicine gelatin sponge particles (RM-GSP, 50 kDa, 135°C heat cross-linkage) soluble at 249 h (10.4 days) in vitro. Common hepatic arteriography was performed before (A), immediately after (B), 1 day after (C), and 2 days after embolization (D). The peripheral branches remained occluded (C, arrow) at 1 day after embolization, and total recanalization of the branches was observed at 2 days after embolization, with no development of collateral pathways.

Table 3 Development of intrahepatic collateral pathways after transcatheter arterial embolization with regenerative medicine Gelatin Sponge Particles (RM-GSP) and Gelpart gelatin sponge particles (G-GSP) in follow-up hepatic angiography

<table>
<thead>
<tr>
<th>Embolic materials</th>
<th>Number of embolized hepatic arteries</th>
<th>Number of hepatic arteries with collateral pathways</th>
<th>Delineation of collateral pathways time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>RM-GSP 50 kDa/135°C</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>RM-GSP 100 kDa/125°C</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>RM-GSP 50 kDa/138 °C</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>G-GSP</td>
<td>4</td>
<td>4</td>
<td>0</td>
</tr>
</tbody>
</table>

kDa kilodalton
Table 4 Distribution of gelatin sponge particles (GSPs) in swine hepatic arteries detected in 60 microscopic visible fields at 100× magnification

<table>
<thead>
<tr>
<th>Embolic materials</th>
<th>Number of GSPs present in hepatic arteries</th>
<th>p value*</th>
<th>Diameter of hepatic artery (mm)</th>
<th>p value**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>&lt;50</td>
<td>50–100</td>
</tr>
<tr>
<td>RM-GSP 50 kDa/135°C</td>
<td>0</td>
<td>&lt;0.001</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>RM-GSP 100 kDa/125°C</td>
<td>0</td>
<td>&lt;0.001</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>RM-GSP 50 kDa/38°C</td>
<td>23</td>
<td>0.043</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>G-GSP</td>
<td>51</td>
<td>-</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

RM-GSP regenerative Medicine gelatin sponge particles, kDa kilodalton, G-GSP Gelport, gelatin sponge particles, NV not value

* Student’s t test was used to compare the number of GSPs between RM-GSP and G-GSP groups; ** χ² test was used to compare the distribution of GSPs between RM-GSP and G-GSP groups

Discussion

In the in vitro study, RM-GSPs were prepared with various solubility times by modulating the temperature of dry heat for sterilization. RM-GSPs with longer solubility times were acquired with higher temperatures, from 110 to 138°C. The RM-GSP dry-heated at 150°C and the G-GSP did not become soluble. Clinically available insoluble GSs should be prepared with cross-linkage at a temperature of more than 150°C, although this detailed information is not widely publicized (personal information provided by the manufacturer, Nihon Kayaku, Tokyo, Japan). The present in vitro study clarified the extent to which heat-induced cross-linkage determines the solubility time of dried GS. The mean soluble time of 100RM-GSP increased more than that of 50RM-GSP at the same temperature. It is considered that the higher molecular weight per unit volume enables easier formation of heat-induced cross-linkage.

The solubility times of 135°C/50RM-GSP, 125°C/100RM-GSP, and 138°C/50RM-GSP in vitro were 57.7 h (2.4 days), 57.6 h (2.4 days), and 249.0 h (10.4 days), respectively, while the recanalization times in vivo ranged from 4 to 24 h, 4 to 24 h, and 24 to 48 h, respectively. The solubility time of RM-GSP was shorter in vivo compared with that in vitro, but we did not investigate the specific reason for this in the present study. We speculate that enzymes to dissolve RM-GSP might exist in the blood and accelerate the soluble time in vivo.

Blood tests and histological examination revealed no adverse effects in the swine. Dry-heat sterilization at 110°C or higher eliminates bacteria and viruses, and RM-GS is considered to be safe in swine. RM-G is extracted from fresh pig skin and is processed to remove endotoxins via ultrafiltration.

In the clinical setting, THAE for HCC causes marked development of intra- and extrahepatic collateral pathways [7, 9, 12]. The development of extrahepatic collateral pathways (e.g., the inferior phrenic artery, intracostal artery, omental artery, and internal mammary artery) is often observed in long-term survivors of HCC treated by repeated THAE [5–11]. The development of intrahepatic collateral pathways is also observed in the case of peripheral occlusion of hepatic arteries. It has been reported that intrahepatic collaterals were usually demonstrated 3 to 4 weeks after embolization with Ivalon particles and gelatin GSPs [9, 10]. Intrahepatic collaterals have been observed as a fine network around previously occluded segmental arteries. Vagal branches originally exist in parallel with the hepatic arteries, supplying the portal triads and their contents [15, 16]. The intrahepatic collaterals probably represent enlarged vaginal branches of the hepatic arteries [9].

In the present study, intrahepatic collateral pathways were confirmed 4 days after THAE with G-GSP. Therefore, GS with a soluble time of less than 4 days is required to inhibit the development of collateral pathways. The recanalization times of branches of the hepatic artery embolized with 135°C/50RM-GSP, 125°C/100RM-GSP, and 138°C/50RM-GSP were 1, 1, and 2 days, respectively. Collateral pathways did not develop in the presence of these embolic materials. Although there are no reports regarding the relation between ischemic term and extent of necrosis for HCC, a longer ischemic period is considered to cause more extensive damage to HCC. In the vivo study, 138°C/50RM-GSP was the soluble embolic material with the longest occlusion time without developing collateral pathways.

Limitations exist in the present experimental study, which was restricted to swine. The magnitude of the soluble time of RM-GSP could be greater or less in the clinical situation in humans. In addition, no report exists to confirm that ischemia with duration of less than 2 days results in sufficient HCC necrosis. In contrast, according to the report of Tanaka et al. [14], follow-up computed tomographic scan after THAE with GSP for HCC demonstrated the gas bubble in the tumor increased greatest at 24 h after THAE followed by 1 h after although no gas bubble was detected on computed tomography immediately after. Ischemic damage to HCC treated by THAE may occur at an earlier stage than we supposed. However, the utility and properties of soluble GSP should be
confirmed by investigating tumor necrosis in an animal tumor model or pilot clinical study [17].

As a subject for future study, soluble RM-GS may have a potential role as a carrier of anticancer drugs for arterial chemotherapy. Anicancer drugs could be intensified at the tumor site for an appropriate time using RM-G with various solubility times.

In conclusion, RM-GSPs with various solubility times were prepared by modulating the temperature from 110 to 138°C. The extent of heat cross-linkage determines the solubility of the RM-GSP. 138°C/50RM-GSP was the soluble GSP with the longest occlusion time and inhibits the development of collateral pathways when THAE is performed.

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References