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Experimental renal and hepatic artery embolization with a new embolic agent, atelocollagen, in a porcine model

Baba Yasutaka, Hayashi Sadao, Ikeda Shunichiro, Higashi Michiyo, Nakajo Masayuki

PURPOSE
We aimed to investigate the potentiality of atelocollagen, a new embolic agent which is collagen type I in a porcine experimental model.

MATERIALS AND METHODS
Three pigs underwent transcatheter embolization of lower interlobular arteries of the renal artery (n=6) and one branch of the hepatic artery (n=3) with collagen type I. Angiography was performed prearterial, during, and postarterial embolization. After the procedure, samples from the embolized organs were evaluated by histological analysis.

RESULTS
Six lower interlobular renal arteries and three hepatic arteries were successfully embolized by administration of 0.8±0.3 mL and 2.9±1.2 mL, respectively, of the collagen type I. Histological findings of the embolized kidney specimens showed that the collagenous materials filled the arterial lumen, whose size ranged from 2.02 to 839.82 μm and reached the level of afferent arteries of glomerular tufts. Although the area of occluded arteries of the liver was smaller than the kidney, histological findings of the liver specimens showed that the collagenous materials filled small arterial lumens from 2.81 to 187.86 μm in diameter.

CONCLUSION
Atelocollagen, a collagen type I, has the potential to be used to embolize the distal vessels of both renal and hepatic arteries.

From the Departments of Radiology (B.Y. ✉ *yasutaka@m3.kufm.kagoshima-u.ac.jp*, H.S., I.S., N.M.), and Human Pathology, Field of Oncology (H.M.), Kagoshima University, Kagoshima, Japan.

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Collagen, the most abundant protein in the human body, is a vital component of the extracellular matrix and connective tissue, including blood vessels. Twenty-nine types of collagen have been identified, seven of which (types I–III, V, XI, XXIV, and XXVII) are fibrillar and assemble as stable triple helices, which then form a complex higher order three-dimensional fibrous superstructure (1–2). Collagen functions in activating platelets through binding the von Willebrand factor and platelet derived collagen receptors (3).

Recent reports have described the use of collagen as an arterial puncture closer device, and treatment for postcatheterization pseudoaneurysm and distal coronary artery perforation (4–6). Collagen has also been used as an embolic material to occlude the intentional vessels in clinical and experimental studies (7–13). Most of these previous reports involve microfibrillar collagen, which is characteristic with long fiber.

The aim of this study was to investigate the efficacy and effects, in terms of tissue permeability, histological changes, and blood flow changes, of hepatic or renal arterial embolization with atelocollagen, which is collagen type I, in a porcine experimental model.

Materials and methods

Procedures

This study protocol was approved by the Institutional Animal Experimental Committee. Three female pigs, each weighing 33 kg, were included in this study. All procedures were performed under general anesthesia. Animals were placed supine, and general anesthesia was administered with an intramuscular injection of ketamine hydrochloride (5 mg/kg Ketalar Intramuscular 500 mg, Daiichi Sankyo, Tokyo, Japan) and medetomidine chloride (80 μg/kg Domitor, Zenoaq, Fukushima, Japan), and maintained with administration of halothane (4% Fluothane, Takeda Pharmaceutical, Osaka, Japan) using a mask. After anesthetic administration, an endotracheal tube was inserted and anesthesia was maintained with halothane (1.5%), nitrous oxide (1.5 L/min), and oxygen (1.5 L/min). Electrocardiography was used to monitor heart rate and rhythm. Oxygen saturation and real-time blood pressure were monitored using a pulse oxymeter (BP-608V, OmronColin, Tokyo, Japan). Vascular sheaths (6 F and 7 F) were inserted into the femoral artery and carotid artery, respectively. A subtraction angiography system (Allura Xper FD20, Philips, Eindhoven, Holland) was used to perform the procedure, and antibiotics were continuously administered throughout the study. After catheterization of bilateral renal arteries, blood flow was measured using FloWire (Volcano Japan, Tokyo, Japan) before and after the procedure. Next, selective catheterization of the lower branch of the renal artery was performed with a 4 F catheter (C2, Hanako, Saitama, Japan). Collagen type I (3% Koken atelocollagen implant, Tokyo, Japan)

(Fig. 1) was injected through the catheter until blood flow stasis was achieved in the lower branch of the renal artery. Repeated renal angiograms were performed (iopamidol 300, Oypalomin 300, Konica Minolta, Tokyo, Japan) at an injection rate of 3 mL/s, with a total injection volume of 6 mL before and after this procedure to check occlusion of the lower pole of renal arteries. A celiac angiogram was performed with a 4 F catheter (C2, Hanako) and sequential hepatic arterial embolization with atelocollagen through the catheter until the blood flow stasis was achieved in the hepatic artery. Repeated celiac angiogram (iopamidol 300, Oypalomin 300, Konica Minolta, Tokyo, Japan), at an injection rate of 4 mL/s with a total injection volume of 8 mL, was performed.

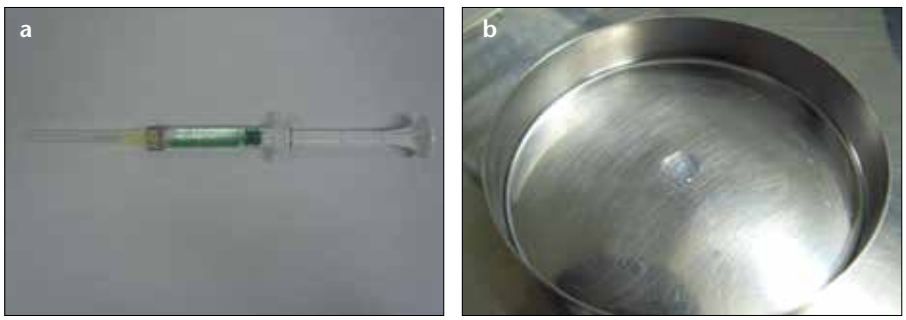

Figure 1. a, b. Atelocollagen is in liquid form at room temperature **(a)**. After warming at 37°C, it becomes a gel **(b)**.

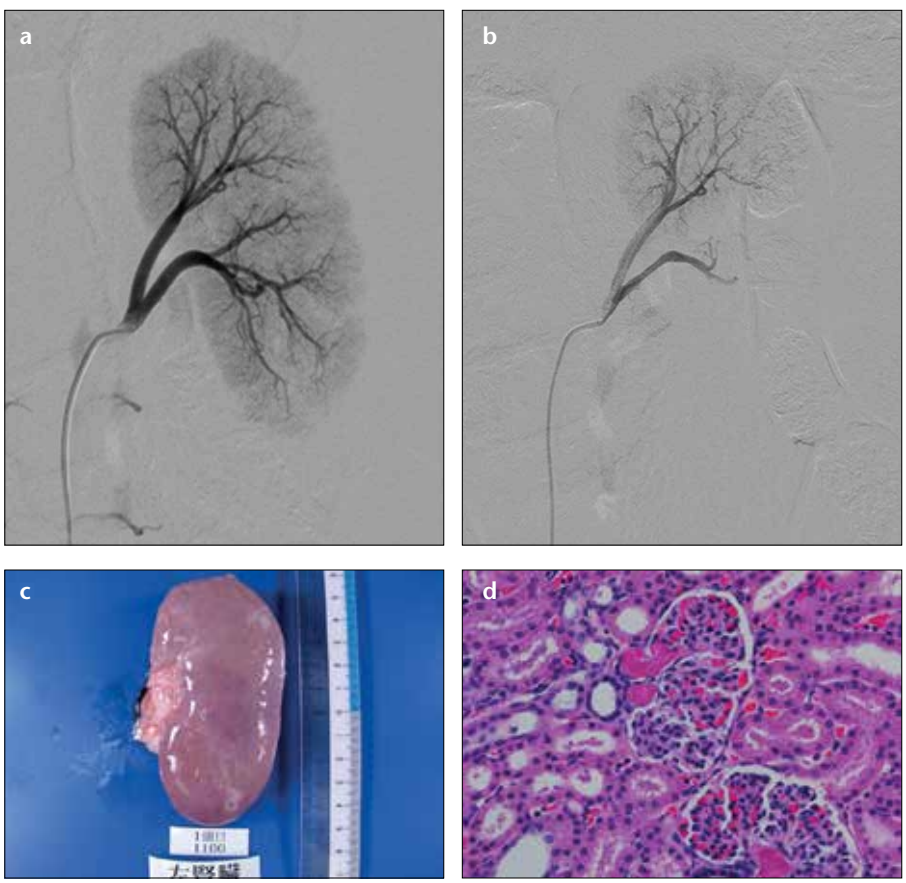

Figure 2. a–d. Renal angiogram **(a)** shows the distribution of the left renal parenchyma. After embolizing the lower branch of the renal artery, renal angiogram shows no visualization of the lower branch of the renal artery **(b)**. Red colored areas of the gross resected specimen of the kidney shows congestion or embolized areas **(c)**. H-E staining (×40) shows the collagenous material within the glomerular tufts **(d)**.

Histological evaluation

After renal embolization, animals were euthanized on the day of study, approximately two hours after transcatheter arterial embolization. Embolized organs were immediately harvested and immersed in buffered 3% formalin liquid fixation for seven days. Specimen samples were cut from paraffin blocks and examined using hematoxylin-eosin (H-E) staining. A pathologist (M.H.) evaluated the pathological slides according to the following considerations: 1) to determine in which periphery levels of the renal arteries the embolic materials were present, and 2) to assess changes of tissue around the renal arteries. Examination was performed with a Nikon Eclipse 80i (Nikon, Tokyo, Japan) and Nikon NIS-elements documentation ver. 3.22 (Nikon).

Results

Six lower interlobular renal arteries and three hepatic arteries were successfully embolized by administration of 0.8±0.3 mL and 2.9±1.2 mL, respectively, of collage type I (Figs. 2, 3).

Histological findings of the embolized vessels are shown in Tables 1 and 2. Histological analysis of kidneys showed that the collagenous materials filled arteriole lumens from 2.02 to 839.82 µm in diameter, and reached the level of afferent arterioles of glomerular tufts (Fig. 2). In the liver, collagenous materials filled arteriole lumens from 2.81 to 187.86 µm in diameter (Fig. 3). No necrotic areas were found in the arterial walls in either organ.

The renal and celiac arterial velocity and flow changed significantly after embolization. In the renal artery group, the maximum peak systolic velocity decreased immediately after embolization (38.6 cm/s vs. 16.7 cm/s, respectively), while an increase was detected in the celiac artery group (31 cm/s vs. 51.3 cm/s, respectively).

Discussion

Atelocollagen is a nearly purified form of type I collagen that lacks high antigenicity, as the non-coil region has been removed by protease treatment (14). It is in liquid form at low temperature, and forms a gel at 37°C (14). Therefore, atelocollagen seems to be a more ideal embolic agent compared to microfibrillar collagen and gelatin sponge.

The concentration of collagen affects the injectability through a needle

Table 1. Diameter of embolized renal arteries in swine

	Diameter of embolized renal artery (μm)		
No.	Median	Standard deviation	Range
Right kidney			
1	8.17	110.96	2.02–839.82
2	9.96	12.8	3.65–84
3	8.87	9.55	3.62–46.96
Left kidney			
1	14.77	40.74	4.18–232.1
2	12.02	17.77	4.18–64.75
3	7.38	36.26	4.18–270.89
Overall	9.61	51.9	2.02–839.82

Table 2. Diameter of embolized hepatic arteries in swine

	Diameter of embolized hepatic artery (μm)		
No.	Median	Standard deviation	Range
1	14.14	16.55	4.3–73.81
2	8.01	19.38	2.81–62.88
3	12.98	24.38	4.18–1887.86
Overall	13.69	20.88	2.81–187.86

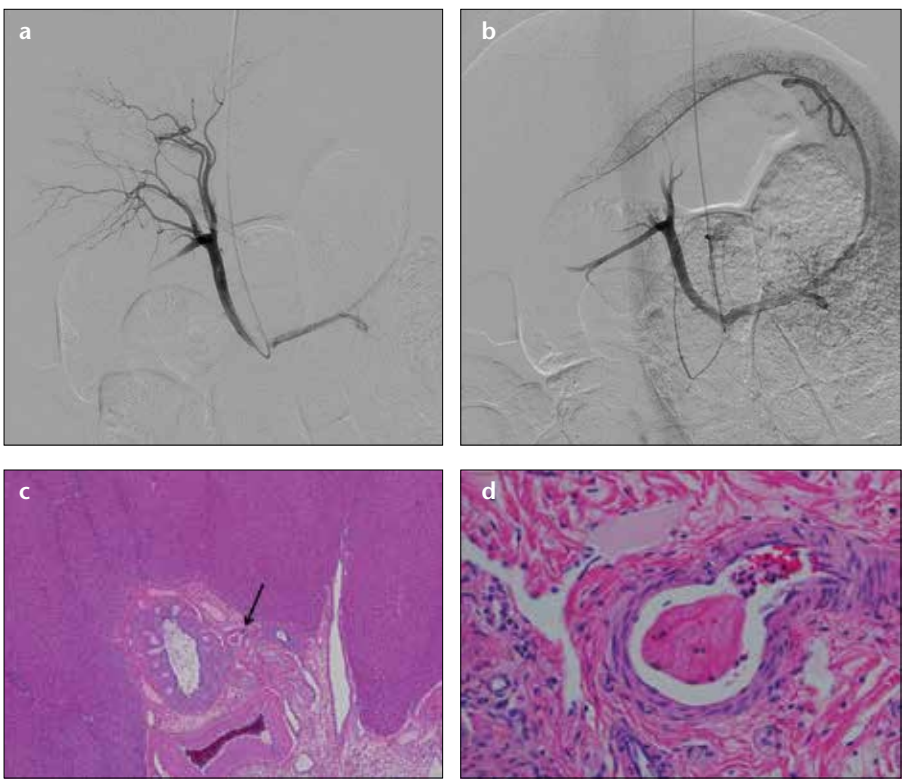

Figure 3. a–d. Celiac angiogram **(a)** shows the distribution of hepatic blood flow. After embolizing the hepatic arteries, celiac angiogram **(b)** shows no visualization of hepatic arteries. H-E staining (×4) shows the collagenous material within the hepatic arteries (*arrow*) and no evidence of inflammatory change within or without vascular wall **(c)**. Magnified view of the H-E staining (×40) shows the collagenous material within the hepatic arteries adjacent to the bile duct **(d)**.

or catheter. Geutjes et al. (15) reported that both 3% and 4% (w/v) collagen suspensions were acceptable concentration levels with respect to the force used (<50 N). In our study, we used a 3% atelocollagen suspension. In our experience with the suspension, we found 3% atelocollagen difficult to inject through a 2 F microcatheter. Therefore, we chose a 4 F diagnostic catheter to inject the 3% atelocollagen without resistance.

Although this was an acute study (animals were sacrificed on the same day of the study), atelocollagen filled not only the main arterioles but also glomerular tufts of the kidney or distal peribiliary hepatic arteries. In addition, there were no changes in inflammation around the hepatic and renal arterioles. In contrast to our results, a previous experimental study using microfibrillar collagen reported granulomatous arteritis (7). However, another study using purified type I bovine tendon collagen showed that it filled arterioles of the kidney with no apparent inflammation around the arterial walls (16). These findings are consistent with those of our study. It is worth noting that this study was performed under laparotomy and direct cannulation into the renal arteries, therefore the method of embolization differs from the transcatheter arterial embolization technique in our study.

In a flow dynamics study after transcatheter arterial embolization (TAE) for hepatocellular carcinoma the peak velocity (PV) of the proximal right hepatic artery (RHA) decreased immediately after TAE of RHA, probably due to the block of the RHA by embolization (17). The authors proposed the possibility that the increased resistance caused the decrease of PV of proximal RHA. In our study, the PV of the renal artery decreased after TAE, but the PV of the celiac artery increased. The reason for this increase could be due to the position of the FloWire tip, which was in the celiac trunk proximal to the branching of the hepatic artery, splenic artery, and gastroepiploic artery. It is likely that the buffer effect after TAE of the hepatic artery could decrease resistance of the celiac artery.

There were some limitations in our study. First, our study was not a follow-up study and did not assess changes of embolized organs over time. Second, we were unable to visualize atelocolla-

gen when embolization was performed under fluoroscopy, as we did not mix it with contrast medium in order to preserve its natural viscosity.

In conclusion, atelocollagen shows potential as an ideal embolic agent because of its low antigenicity, embolus formation in a wide range of size, and lack of inflammatory response.

Conflict of interest disclosure

Baba Yasutaka received diagnostic catheters from Boston Scientific Japan. The remaining authors had no conflicts of interest.

References

1. Herr AB, Farndale RW. Structural insights into the interactions between platelet receptors and fibrillar collagen. J Biol Chem 2009; 284:19781–19785. [CrossRef]
2. Koide T. Application of collagen-like triple-helical peptides to biochemical studies elucidating the collagen structure and functions. Seikagaku 2010; 82:474–483.
3. Nieswandt B, Watson SP. Platelet-collagen interaction: is GPVI the central receptor? Blood 2003; 102:449–461. [CrossRef]
4. Koreny M, Riedmuller E, Nikfardjam M, Siostrzonek P, Mullner M. Arterial puncture closing devices compared with standard manual compression after cardiac catheterization: systematic review and meta-analysis. JAMA 2004; 291:350–357. [CrossRef]
5. Hamraoui K, Ernst SM, van Dessel PF, et al. Efficacy and safety of percutaneous treatment of iatrogenic femoral artery pseudoaneurysm by biodegradable collagen injection. J Am Coll Cardiol 2002; 39:1297–1304. [CrossRef]
6. Aleong G, Jimenez-Quevedo P, Alfonso F. Collagen embolization for the successful treatment of a distal coronary artery perforation. Catheter Cardiovasc Interv 2009; 73:332–335. [CrossRef]
7. Kaufman SL, Strandberg JD, Barth KH, White RI Jr. Transcatheter embolization with microfibrillar collagen in swine. Invest Radiol 1978; 13:200–204. [CrossRef]
8. Diamond NG, Casarella WJ, Bachman DM, Wolff M. Microfibrillar collagen hemostat: a new transcatheter embolization agent. Radiology 1979; 133:775–779.
9. Kumar AJ, Kaufman SL, Patt J, Posey JB, Maxwell DD, White RI Jr. Preoperative embolization of hypervascular head and neck neoplasms using microfibrillar collagen. AJNR Am J Neuroradiol 1982; 3:163–168.
10. Daniels JR, Kerlan RK Jr, Dodds L, et al. Peripheral hepatic arterial embolization with crosslinked collagen fibers. Invest Radiol 1987; 22:126–131. [CrossRef]
11. Daniels JR, Sternlicht M, Daniels AM. Collagen chemoembolization: pharmacokinetics and tissue tolerance of cis-diamminedichloroplatinum(II) in porcine liver and rabbit kidney. Cancer Res 1988; 48:2446–2450.
12. Quinn SF, Frau DM, Saff GN, et al. Neurologic complications of pelvic intraarterial chemoembolization performed with collagen material and cisplatin. Radiology 1988; 167:55–57.
13. Cho KJ, Fanders B, Smid A, McLaughlin P. Experimental hepatic artery embolization with a collagen embolic agent in rabbits. A microcirculatory study. Invest Radiol 1989; 24:371–374. [CrossRef]
14. Helseth DL Jr, Veis A. Collagen self-assembly in vitro. Differentiating specific telopeptide-dependent interactions using selective enzyme modification and the addition of free amino telopeptide. J Biol Chem 1981; 256:7118–7128.
15. Geutjes PJ, van der Vliet JA, Faraj KA, et al. Preparation and characterization of injectable fibrillar type I collagen and evaluation for pseudoaneurysm treatment in a pig model. J Vasc Surg 2010; 52:1330–1338. [CrossRef]
16. Riccetto CL, Novaski GL, Palma PC, Dambros M, Thiel M, Vidal Bde C. New biocompatible highly purified collagen gel for therapeutic renal artery embolization. Urology 2006; 67:851–854. [CrossRef]
17. Taourel P, Dauzat M, Lafortune M, Pradel J, Rossi M, Bruel JM. Hemodynamic changes after transcatheter arterial embolization of hepatocellular carcinomas. Radiology 1994; 191:189–192.