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A Light-microscopic Observation of Rabbit Auricular Artery Embolization with Gelatin Sponge Powder

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ABSTRACT. Rabbit auricular arteries were embolized with gelatin sponge powder. A suspension of less than 0.5 ml was required when injection into the auricular artery. Embolic lesions were distinguishable by palpation and inspection. Gelatin sponge was seen in arterial lumina of 500-1000 μm in diameter, and was absorbed in about one month mainly by phagocytosis of histiocytes. The inner coat of the artery disappeared just after embolization. Endothelial cells proliferated into the thrombus from the 7th day, and then formed a new vascular canal in one to two months, accompanied by a thick inner coat mainly composed of smooth muscle cells. Ischemic necrosis did not occur.

Gelatin sponge was developed as an absorbable hemostatic substance.[1-3] In recent years, it has been utilized as an embolic agent for hepatic tumors.[4] We have attempted to induce a new model of immune complex arteritis on the rabbit auricular artery by means of embolization with gelatin sponge powder saturated with antigen solution.[5] As a preliminary study, rabbit auricular arteries were embolized with gelatin sponge powder only. The proper procedure for this embolization was examined and light-microscopic observations were presented in this report.

MATERIALS AND METHODS

Gelatin sponge (Spongel®) was pulverized with a file and saturated with phosphate buffered saline | PBS | (pH 7.2). The sponge immediately swelled and, while it could pass through a 22 gauge needle, it could not pass through needles of under 25 gauge.

Albino rabbits, weighing 2.5～3.5 kg, were used. The intermediate branches of the caudal auricular arteries, which run through the median line of the rabbit's auricle, were embolized as shown in Fig. 1. After shaving the auricule, suspensions of gelatin sponge powder were percutaneously injected into arteries through 22 gauge needles. Three arteries from two animals were embolized with suspensions of 10～20 mg gelatin sponge powder in 1.0～1.5 ml of PBS. Seven arteries from four animals were embolized with suspensions of 2～10 mg of gelatin sponge powder in 0.2～0.5 ml PBS. These procedures were carried out aseptically. Biopsy specimens were taken from the embolic lesions: seven

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samples at 1～24 hours after embolization, three at 1～3 days after, five at 1～9 weeks after. These were embedded in paraffin and stained with hematoxyline-eosin.

RESULTS

Two animals, which were embolized with the suspensions of 10～20 mg of gelatin sponge powder in 1.0～1.5 ml of PBS, had apoplectic strokes just after injection and died within one day. The other animals, which were embolized with the suspensions of 2～10 mg of gelatin sponge powder in 0.2～0.5 ml of PBS, experienced no difficulties and continued to be observed.

Macroscopic findings:

Immediately after embolization, ischemic changes became visible on the arteries themselves and on the skin taken from the distal side of the intraarterial injections. Several minutes later, the venous plexus dilated and became congested with blood. The embolic lesions of the arteries, which usually occurred in both the truncus and branches at points of intersection as shown in Fig. 1, remained ischemic and palpably hard. A congestive state continued for 3～5 days and was followed by a slightly anemic condition. The embolic lesions also became localized and palpably harder. Between the 3rd～5th week, the arteries became gradually elastic again, and blood flows became visible. Eight to nine weeks after embolization, the skins and arteries returned to their normal state. Ischemic necrosis did not occur.

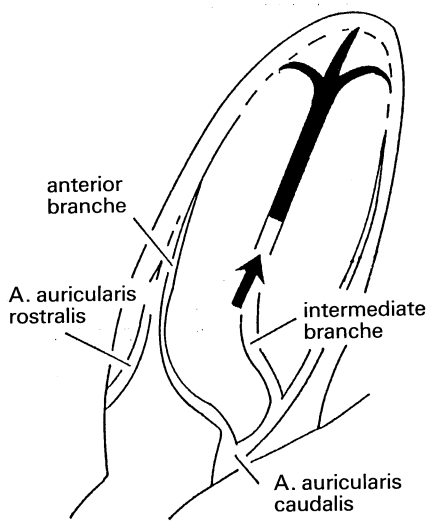

Fig. 1. The scheme for embolization of the rabbit auricular artery. An arrow shows the injection point. The embolic lesion is present as a closed artery.

Light-microscopic finding:

Gelatin sponge could be seen in small arteries having lumina of 500～1000 μm in diameter, but was not seen in capillarier or venules. Four hours

to three days after embolization, the arterial lumina were found to be occupied by gelatin sponge well stained with hematoxyline and showing a cavernous structure containing erythrocytes and polymorphonuclear | PMN | leucocytes (Fig. 2). The inner coat of most lesions had disappeared, but the internal elastic membrane was intact. Some number of PMN leucocytes had infiltrated into the arterial walls. Severe edema and cell infiltrations of both histiocytes and PMN leucocytes were widespread in the dermis around the embolized arteries.

One to two weeks after embolization, the sponge's cavernous structure began to lose and be replaced by a thrombus. Endothelial cells reappeared within the thrombus. In addition there were also many fibroblasts and a large amount of mucinous materials between the collagen bundles of the dermis (Fig. 3). These inflammatory cell infiltrations appeared maximally for one to two weeks, and then decreased

Three to four weeks after embolization, the dermis showed a normal state without any inflammatory reactions. Many small vascular canals, which were surrounded by a single layer of endothelial cells, appeared in lumina occupied by both phagocytic histiocytes and smooth muscle cells (Figs. 4, 5). The gelatin sponge and thrombus may have been phagocytosed by histiocytes. During the ninth week, the embolized arteries exhibited one lumen with a thick inner coat composed of smooth muscle cells (Fig. 6).

Histological investigations did not show any destruction of the arteries or necrosis of the skin.

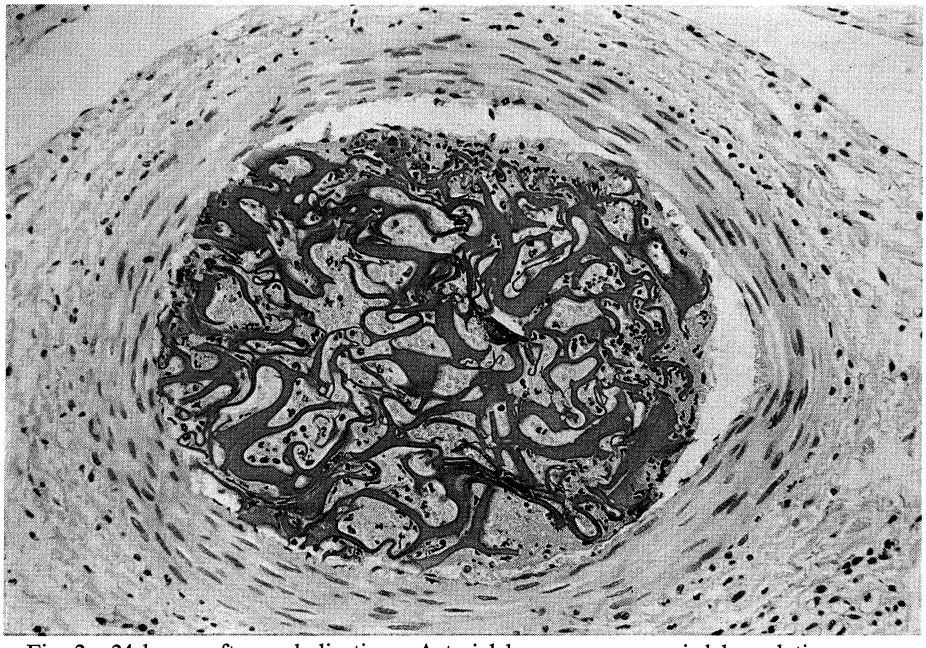

Fig. 2. 24 hours after embolization. Arterial lumen was occupied by gelatin sponge. The inner coat had disappeared and some number of PMN leucocytes had infiltrated. H-E staining, original magnification 200×

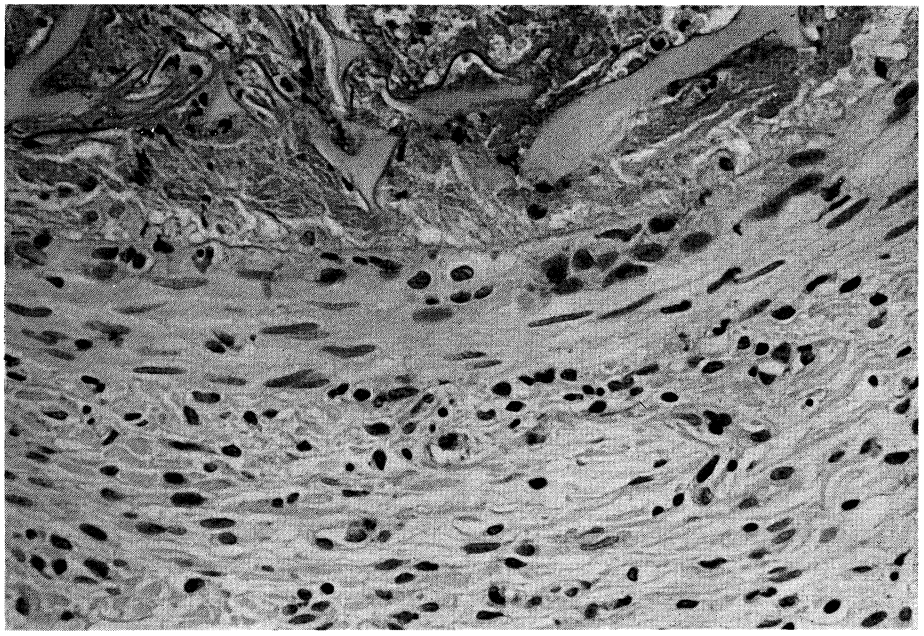

Fig. 3. Two weeks after embolization. Endothelial cells proliferated into the thrombus. Many fibroblasts and histiocytes were seen in the dermis. H-E staining, original magnification 400×

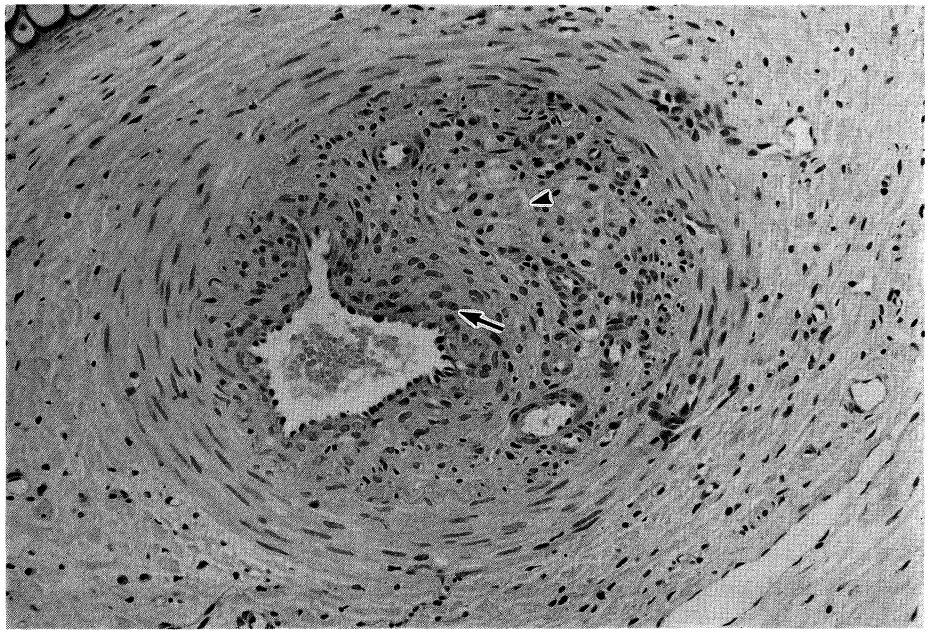

Fig. 4. Three weeks after embolization. Small vascular canals appeared in lumen occupied by smooth muscle cells (arrow) and histiocytes (arrow head). H-E staining, original magnification 200×

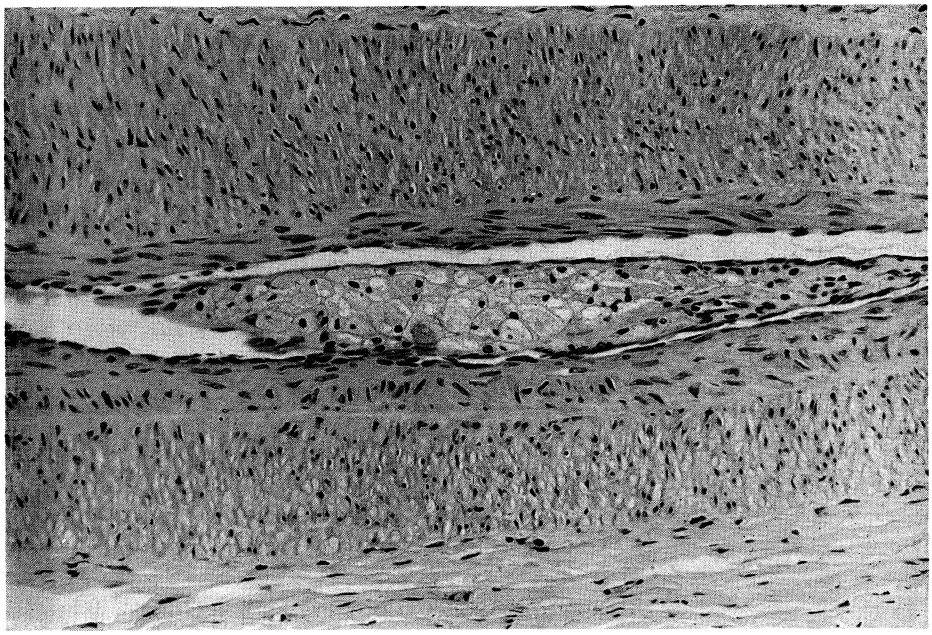

Fig. 5. Five weeks after embolization, longitudinal-cut section. A regenerated inner coat is present. H-E staining, original magnification 200×

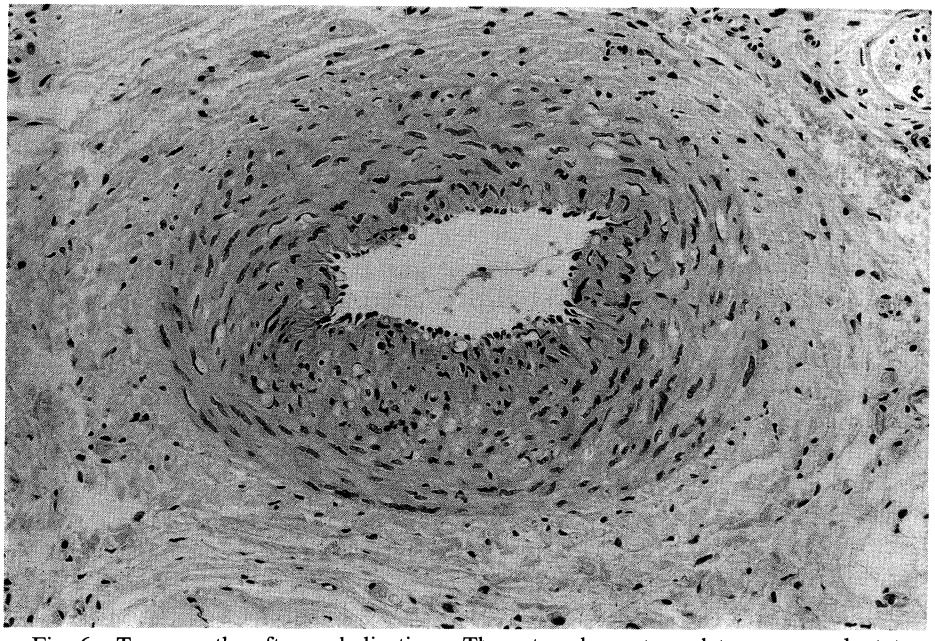

Fig. 6. Two months after embolization. The artery has returned to a normal state with a thick inner coat composed of smooth muscle cells. H-E staining, original magnification 200×

DISCUSSION

As to the cause of the apoplectic stroke which occurred in two of the animals embolized with a suspension of more than 1.0 ml of gelatin sponge, it is considered that the gelatin sponge powder flowed backward and occluded the cerebral arteries. Furthermore, since the powder never passed through capillaries, the dosage of suspension must have been too great to stay only in the auricular artery. Therefore a suspension of less than 0.5 ml should be considered an adequate dosage when injected into the rabbit auricular artery.

Histological studies on the absorbability of gelatin sponge have been carried out by Correll *et al.*[1] and Jenkins & Clarke.[2] These reports showed that gelatin sponge was absorbed in about one month, mainly by the phagocytosis of macrophages, when implanted in the liver, kidney, spleen, omentum, abdominal wall[2] or muscle.[1] The cellular response appeared to become maximal in 10 to 25 days, and then recedes. In some instances, where there were many PMN leucocytes, gelatin sponge disappeared more rapidly.[2] In our observations, gelatin sponge began to liquefy and lose its cavernous structure from the 7th day after intraarterial injections. Then the gelatin was phagocytosed by histiocytes, after which it disappeared from arterial lumina in about two to three weeks.

In an investigation of experimental hepatic artery embolization with gelatin sponge carried out by Cho & Lunderquist,[4] the following observations were made. The size of the occluded vessels did not correspond to the sizes of the gelatin sponge particles, this is because the particles swell when saturated with water, because they are also compressible and because they become deformed when injected into an artery. In our study, sponge particles could be seen only in small arteries having lumina of 500$\sim$1000 μm in diameter. Cho & Lunderquist's radiological observations suggested that there was a tendency for recanalization. Our results supported their work and revealed histologically the process of recanalization. The inner coat of the arterial wall disappeared just after embolization. Endothelial cells proliferated into the thrombus and formed new vascular canals. The regenerated inner coat was mainly composed of smooth muscle cells which may have come from middle coat. With regard to the alteration of arterial walls in the repair mechanism, similar findings have been presented in a study of mechanical injury using Fogarty's catheter.[6] The altered artery has been investigated as a model for atherosclerosis by many researchers.[7]

Ischemic necrosis did not occur in this study because the rabbit auricule is abundant in arterioloanastomosis. Since a proper dosage of gelatin sponge emboli did not cause death of the animals, long term follow-up studies may be possible. Biopsy samples could be taken easily and with certainty because the embolized lesions distinguishable by palpation and inspection. Gelatin sponge is saturated with a solution and absorbed in the tissue. For the above reasons, promising arteritis experiments using gelatin sponge and the auricular artery of the rabbit are expected.

Acknowledgment

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