Original Article

In vivo microscopy of arterial distribution embolic particles in rabbit mesenteric artery

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ABSTRACT

Purpose: To study the arterial distribution of embosphere microsphere (EM) and polyvinyl alcohol (PVA) particles in rabbit mesenteric artery using *in vivo* microscopy.

Methods: Sixteen New Zealand rabbits were divided into four groups, namely large PVA (560–710 μm), small PVA (150–350 μm), large EM (500–700 μm), and small EM (100–300 μm). The mesenteric arteries of the experimental animals were embolized under fluoroscopic guidance and visualized using *in vivo* microscopy. The embolized vessel diameter and arterial distribution of embolic agents were compared.

Results: The diameters of occluded vessels in large PVA, small PVA, large EM, and small EM groups were 430.60 ± 67.30, 200.95 ± 70.54, 387.79 ± 92.51, and 143.81 ± 39.65 μm, respectively. PVA occluded significantly larger vessels than EM when the particle size was similar ($P < 0.001$). The proportion of EM at the bifurcation of the artery was significantly higher than that of PVA particles (large PVA < large EM, $\chi^2 = 4.325$, $P < 0.038$; small PVA < small EM, $\chi^2 = 6.68$, $P < 0.01$).

Conclusion: Both PVA and EM could occlude vessels smaller than the particle size, and EM resulted in deeper penetration. The location of embolic particles in the artery is mainly related to the shape of particles.

KEY WORDS: Animal experiment, arterial distribution, embolic particle, embolization treatment, microscope

INTRODUCTION

Transcatheter embolization has developed into one of the most important techniques for interventional radiology.[1] With the advent of microcatheters and new embolic materials, interventional embolization has been widely applied in tumor therapy and gastrointestinal bleeding.[2,3] The basic goal of embolization is to block, reduce, or slow blood flow.[4] Imaging examinations are often used to evaluate the distribution of embolic agents,[5-7] and histopathology analysis is another commonly used method.[8] In this study, we described our preliminary findings of the size of the occluded vessels and the distribution of embolic particles in rabbit mesenteric artery using *in vivo* microscopy.

MATERIALS AND METHODS

In vivo microscope

The *in vivo* microscope is specialized equipment for observing dynamic microcirculation. It is modified from the traditional microscope, and its light can pass through tissues using a high-throughput light microscope with long focal length. After anesthesia, the organs or tissues of the experimental animal are placed on the observation window and viewed through an objective lens.[9]

Embolic particles

The embolic agents used in this study were nonspherical polyvinyl alcohol particles (PVAs; Alicon, Hangzhou, China) and embosphere microspheres (EMs; Biosphere Medical, Rockland, USA). Both PVA and EM are commonly used as permanent embolic agents in the management of arteriovenous malformations and hypervascular tumors. There were two granulometric size ranges of PVA, 560–710 μm [large PVA, Figure 1a] and 150–350 μm [small PVA, Figure 1b and c]), compared

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with similar size ranges of EM, 500–700 μm [large EM, Figure 1d] and 100–300 μm [small EM, Figure 1e and f].

Experimental animals

All experiments in our study were approved by the Institutional Animal Care and Use Board of our institution and were performed according to facility regulations on animal care and experiments. Sixteen adult New Zealand white rabbits, each weighing 2.5–3.0 kg, were divided into four groups of four rabbits each.

Embolization procedure

All animals were not fed for 12 h before operation and anesthetized with intramuscular injection of xylazine hydrochloride (Shengda Animal Medicine, Dunhua, China) at a dose of 0.2 mL/kg. After sterilizing the left groin region and skin cutting, the left femoral artery was isolated, and a self-made sheath was introduced. Routine angiography was performed before embolization with a 2.7 F microcatheter (Progreat; Terumo Corporation, Tokyo, Japan) to confirm the anatomy of mesenteric artery. For angiography, 3 mL of contrast medium (Ioversol; Hengrui Pharma, China) was injected at a rate of 1 mL/s and using pressure of 300 pounds per square inch. Then, the branches of the mesenteric artery, which were the feeding arteries of the jejunum and ileum, were superselectively catheterized.

The suspensions of PVA and EM were prepared as previously described. Briefly, one bottle of embolic particles was poured into a 20-mL syringe. Then, PVA particles were compressed to remove the air, and the supernatant fluid of EM was expressed through the tip. After that, the syringe was filled to 20-mL total volume with a 1:1 saline and contrast mixture. For each embolic agent, the total 20-mL suspension was divided into ten 2-mL syringes after agitation. Afterward, the suspension was delivered into the feeding arteries of the jejunum and ileum at a rate of 0.1 mL/s via the microcatheter with a 2-mL syringe, each of which was followed by a 5-mL saline flush. Approximately 6 mL of suspension was injected in each animal.

Microscopic examination

A standard compound binocular microscope (NK200; Nanguang, Suzhou, China) was modified for *in vivo* microscopy and equipped for transillumination and epi-illumination. After embolization, animals were transferred to the laboratory. If necessary, xylazine hydrochloride was intramuscularly injected at the dose of 0.1 mL/kg. After sterilizing, experimental animals underwent a midline abdominal incision. The procedures of *in vivo* microscopy have been described in previous studies.[10] The jejunum and ileum were gently exteriorized through the incision and positioned over a window of mica on a specially designed microscope tray. Throughout the observation period, the jejunum, ileum, and mesenteric region were constantly irrigated with normal saline at body temperature. The microscopic images were obtained using ×4, ×8, ×10, ×20, and ×40 dry objectives and ×10 oculars, and the video images were recorded and stored in the computer connected to the microscope.

To evaluate the location of embolic agents and the diameter of occluded vessels, images of the occluded vessels were recorded and stored in the computer for further measurement. In our study, the positions of embolic agents in the arteries were divided into the point of bifurcations and side branches of vessels. The diameter of vessels was measured as the internal diameter of the vessel lumen. Furthermore, both the proximal and distal diameters of occluded vessels were measured at a point where they were 0.5–1.0 mm away from the particle, which is in relation to the size of the particles.

Statistical analysis

Quantitative data were expressed as means ± standard deviation. Mann–Whitney and Kruskal–Wallis tests were

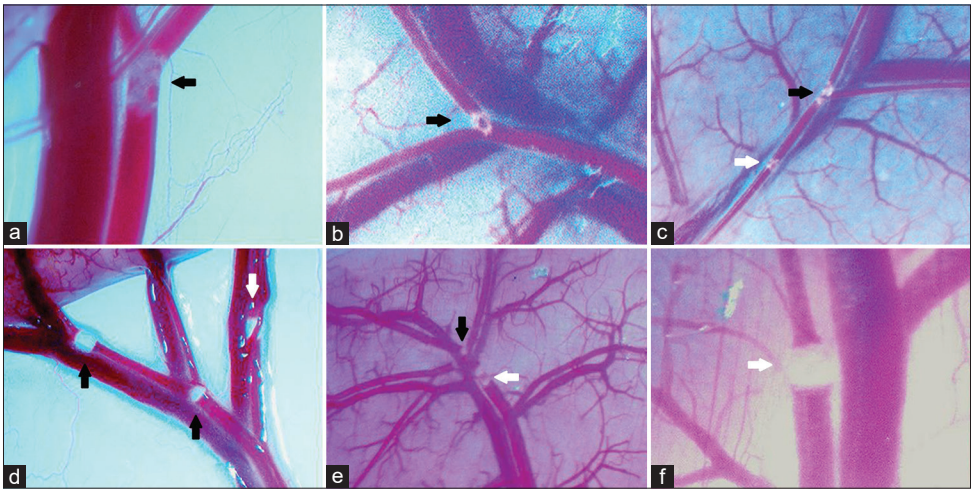

Figure 1: Embolic agents in rabbit mesenteric artery (a) a large PVA particle at the bifurcation of the artery; (b and c) small PVA particle in the artery; (d) large microspheres at the bifurcation of the artery; (e and f) small EMs at the bifurcation of the artery. PVA = Polyvinyl alcohol, EM = Embosphere microsphere

performed for comparisons of the diameter of the occluded vessels. A Chi-square test was performed to compare the location of occlusion between each type of microsphere. Statistical significance was set at $P < 0.05$ (two-sided). The data were analyzed with SPSS statistical software (version 25.0; IBM Corporation, Armonk, NY, USA).

RESULTS

Observed embolic agent

A total of 715 embolic agents were observed and recorded using an *in vivo* microscope. The number of embolic agents in each group is shown in Table 1.

Diameter of occluded vessel

The diameter of occluded vessels was 430.60 ± 67.30 μm (median 440.34 μm) in large PVA group, 200.95 ± 70.54 μm (median 175.05 μm) in small PVA group, 387.79 ± 92.51 μm (median 376.16 μm) in large EM group, and 143.81 ± 39.65 μm (median 138.65 μm) in small EM group.

For the same kind of embolic agents, large agents occluded significantly larger vessels than small particles (both $P < 0.001$). For agents with similar size, nonspherical PVA particles occluded significantly larger vessels than spherical EM (both $P < 0.001$) [Figure 2].

Location of embolic agent

The location of embolic agents was observed with a low-power microscope. In general, 527 (73.71%) embolic agents were located at the bifurcation of the artery [Figure 1a-e, black arrows], and 188 (26.29%) embolic agents were located at the side branch of vessels [Figure 1c, e, and f, white arrows].

For the same kind of embolic agents, the proportion of large agents located at the bifurcation of the artery was higher than that of small agents but with no statistical significance. For agents of similar size, the proportion of spherical EM at the bifurcation of the artery was significantly higher than that of nonspherical PVA particles [Table 1].

DISCUSSION

In clinical practice, transcatheter embolization is now widely used in the management of many kinds of disease, especially for the treatment of hepatocellular carcinoma.[11,12] However, in most situations, interventional radiologists have to rely on their own clinical experience when choosing the embolic agent. The results of our study showed that both PVA and EM could occlude vessels much smaller than the particle size. In addition, most of these embolic particles were located at the bifurcation of artery, which, to our knowledge, had not been reported in previous studies. Compared to PVA particles, EMs occluded significantly smaller vessels when their particle size was similar ($P < 0.001$), and PVA particles were more likely to be located at the bifurcation, which had not been described in previous studies.

Several animal studies were conducted to evaluate the arterial distribution of embolic agents and the size of occluded vessels.[6,13] However, most of these studies were based on the imaging and histological findings. In our study, we used an *in vivo* microscope that allowed direct observation of embolic particles. The embolic particles in our study were embolic agents commonly used in clinical practice. Since these embolic agents are not a precise size but within a granulometric size range, we decided to use those embolic agents with significantly different granulometric size ranges.

Our findings were quite different from those in previous experiments. With the application of *in vivo* microscopy, the location of embolic particles could be directly observed, as well as the diameter of occluded vessels. Interestingly, we found that the diameter of distal part of embolic agents in the vessel was much smaller than the granulometric size of embolic particles, and in most cases, the diameter of proximal area of embolic agents was similar or smaller than the

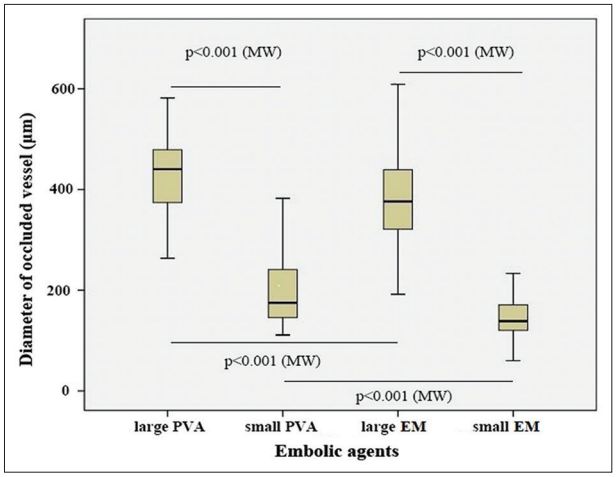

Figure 2: Comparison of the diameter of the vessels occluded by different embolic agents. MW = Mann–Whitney U-test, PVA = Polyvinyl alcohol, EM = Embosphere microsphere

Table 1: Comparison of the proportion of location according to the kind and size range of embolic agents

Location	PVA		EM		Large embolic agents		Small embolic agents	
	Large (156)	Small (169)	Large (168)	Small (222)	PVA (156)	EM (168)	PVA (169)	EM (222)
Bifurcation	112 (71.79)	111 (65.68)	137 (81.55)	172 (77.48)	112 (71.79)	137 (81.55)	111 (65.68)	172 (77.48)
Side branch	44 (28.21)	58 (34.32)	31 (18.45)	50 (22.52)	44 (28.21)	31 (18.45)	58 (34.32)	50 (22.52)
χ^2, P	1.408, 0.235		0.963, 0.327		4.325, 0.038		6.68, 0.01	

PVA=Polyvinyl alcohol, EM=Embosphere microsphere

granulometric size of embolic particle [Figure 1f]. To reduce error, the diameter of occluded vessels was calculated as the mean of the proximal diameter and the diameter of the distal branches of occluded vessels.

In earlier studies, some scholars believed that PVA particles would remain in vessels similar to their own size.[14,15] The possible reasons for the different results between our study and previous studies are as follows. First, the target organs were different in our study. Second, histologic findings were usually based on the section with embolic particles while ignoring those sections without embolic particles, whereas *in vivo* microscopy allows for the vessels and embolic agents to be directly observed. Third, PVA particles are nonspherical and easier to aggregate with each other, leading to the formation of larger particles. Regarding the location of embolic particles, the proportion of embolic agents at the bifurcation of the artery might be mainly related to the shape of embolic agents. We assumed that PVA particles that are nonspherical might result in increased friction between PVA particles and vessel walls, which might make PVA particles more resistant to moving forward to the next bifurcation.

Based on these findings, it is important to recognize that the embolic particles could have penetrated deeper than we thought. In clinical practice, interventional radiologists should pay more attention to the choice of optimal embolic particles, according to the size of the target vessels. Our study has several limitations. First, the results of our experiments in rabbits cannot be translated directly to humans due to the differences of the patterns and the diameters of arteries between humans and rabbits. Second, the long-term effects of embolization could not be evaluated because the animals were sacrificed immediately after *in vivo* microscopy. Third, only two size ranges of each kind of embolic agent were examined in our study. Fourth, only the mesenteric artery was studied in our experiments, since it can be easily observed using an *in vivo* microscope. However, the embolized vessel diameter may differ in different organs.[15] Fifth, the range of ischemic changes was not observed in the present study, and further studies regarding this matter should be performed.

CONCLUSION

In vivo microscopy shows promise for comparative studies of embolic agents. Both PVA and EM could occlude the vessels smaller than the particle size, and when compared with nonspherical PVA particles, spherical EMs resulted in deeper penetration. In addition, the location of embolic particles in the artery is mainly related to the shape of particles. Moreover, further investigations in different organs with different embolic agents are needed.

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Conflicts of interest

There are no conflicts of interest.

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