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Extravascular Injection of Sclerotic Agents does not Affect Vessels in the Rat: Experimental Implications for Percutaneous Sclerotherapy of Arteriovenous Malformations

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WHAT THIS PAPER ADDS

- Our experimental study demonstrated that extravascular administration of sclerotic agents shows no detrimental influences on vessels first. The results implicated the importance of catheterisation during sclerotherapy for vascular malformations.

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ABSTRACT

Objectives: Sclerotherapy is useful for the treatment of arteriovenous vascular malformations. However, intravascular administration of sclerotic agents into small arteriovenous niduses is often difficult. Extravascular administration of sclerotic agents causes reduction of vascular flow on Doppler echo during clinical sclerotherapy. Therefore, we aimed to investigate whether the extravascular injection of sclerotic agents affects tiny vessels.
Design: Animal study.
Materials: The effect of extravascular injection of sclerotic agents on vessels was investigated using rat femoral and superficial inferior epigastric vessels.
Methods: After surgical exposure of vessels, absolute ethanol, 5% ethanolamine oleate and 3% polidocanol were injected into perivascular surrounding tissues, and their effect on vessels was evaluated after 14 days using histology and coloured silicone rubber injection.
Results: The integrity of the vascular lumen, endothelial cells and vascular patency were not affected by injection of sclerotic agents.
Conclusions: Attenuation of vascular flow of an arteriovenous shunt after extravascular injection of sclerotic agents is transient and/or trivial and does not cause disruption of vessels. Therefore, sclerotic agents should be delivered to obtain sufficient destruction of arteriovenous malformation lesions and blood flow.
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Among various types of congenital vascular malformations, arteriovenous malformations (AVMs) are featured by a wide range of clinical presentations and unpredictable courses, making their treatment difficult. Surgical eradication of arteriovenous niduses of an AVM offers the possibility of complete cure. However, it is often difficult or impossible because of excessive bleeding, resulting in severe morbidities in appearance as well as function, especially in the head and neck.[1–3] Therefore, embolisation and/or percutaneous sclerotherapy are often employed as independent therapies or as adjunct therapies with surgical resection.

During these approaches, a decrease in vascular flow of lesions caused by proximal blockage of arteries supplying AVMs can result in the recurrence of lesions being nourished by much more complicated collateral feeding arteries.[1,4,5] Moreover, re-feeding of lesions after proximal blockage even enhances the expansion of original lesions in a short period of time.[1,4,5] Therefore, during percutaneous sclerotherapy for AVMs, it is necessary to inject sclerotic agents into tiny arteriovenous shunting vessels.[1,6] However, catheterisation into tiny shunting vessels is often technically difficult. In our experiences of Doppler-ultrasonography-

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guided sclerotherapy for AVMs, we have found that unintentional extravascular instillation of sclerotic agents caused attenuation of vascular flow of arteriovenous shunts, although it was unclear if the attenuation was actually caused by damage to vessels and whether it resulted in reduction of AVMs.

Herein, we are interested in whether extravascularly injected sclerotic agents affect tiny vessels. As far as we know, detrimental influences of intravascularly administrated sclerotic agents on vessels,[7–10] extravascular surrounding tissues such as nerve, muscle[8] and skin[11] and effects of extravascularly administrated sclerotic agents on muscle and nerve[12] have been previously reported. However, no reports have described the effect of extravascularly administered sclerotic agents on vessels.

To investigate the detrimental influences of extravascularly injected sclerotic agents on tiny vessels, we employed arteries and veins of rat groin fossa as an experimental model. Following injection of sclerotic agents, their effects on vessels, especially on patency, were evaluated with histology and coloured silicone rubber injection.

Materials and Methods

Experimental model

Wistar rats weighing 300–350 g were used. All the operative procedures were performed under general anaesthesia with intraperitoneal administration of 50 mg kg^{-1} pentobarbital sodium. The study was designed in accordance with the Laboratory Animal Guidelines of Kyorin University School of Medicine.

Right femoral vessels and superficial inferior epigastric (SIE) vessels of the rat were employed to investigate the effects of extravascularly administered sclerotic agents on vessels. The subject vessels were easily identified through an inguinal incision without detachment of transparent surrounding tissues (Fig. 1). After identification of the anterior surface of vessels, 1 ml of sclerotic agents was injected with a 30-G fine needle into the surrounding tissues close to the vessels.

Sclerotic agents

Absolute ethanol (ET), 5% ethanolamine oleate (EO) (composed of a 1:1 mixture of 10% EO (Oldamin®, Fuji Chemical Industry Co., Toyama, Japan) and pure water) and 3% polidocanol (PL) (Polidocasclerol, Zeria Pharmaceutical Co., Ltd., Tokyo, Japan) were used as sclerotic agents because these are known as strong sclerotic agents used for the percutaneous sclerotherapy of vascular malformations.[6,8,11,12]

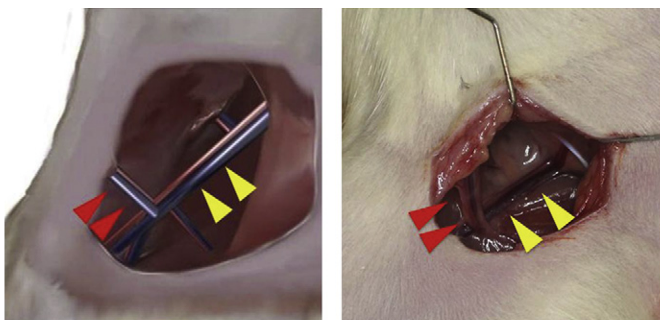

Figure 1. Schematic description of the femoral vessels and superficial inferior epigastric vessels in the rat. The anterior part of the femoral vessels and superficial inferior epigastric vessels are minimally dissected to identify the vessels through an inguinal incision. Vessels are easily viewed through transparent surrounding tissues without detachment of surrounding tissues. Yellow arrowheads indicate the femoral vessels and red arrowheads indicate the superficial inferior epigastric vessels.

Experimental design

A total of 36 rats were allotted to six groups ($n = 6$ for each group). Three groups of rats were used to investigate the effect of ET, EO and PL on femoral vessels and the other three groups were used to investigate the effect of ET, EO and PL on SIE vessels. No specific control was used for the current study because of familiarity with the animals and assays used.[8,13,14] No specific blinding was assigned because we considered that observational bias would not affect the results owing to clarity of the end point. During the experiment, seven animals died, possibly because of overdosage of anaesthesia, and these animals were replaced.

The effect of sclerotic agents on vessels

Two weeks after injection, vessels were inspected through inguinal incision. After careful inspection of the patency of vessels, segments of the vessels and extravascular surrounding tissues were obtained for pathological inspection in four rats for each experimental group. In two rats in each group, 1 ml of yellow-coloured silicone rubber (Microfil; Flow Tech, Inc., Carver, Mass.) was injected through the proximal end of the femoral vessels for the confirmation of vascular patency.

Results

Changes immediately after the injection of sclerotic agents

With injection of ET, the colour of transparent surrounding tissues turned immediately to non-translucent white, while EO and PL did not cause any discolouration with injection. Dilated veins appeared attenuated in patches with the pressure of pooled sclerotic agents. However, none of the segments showed complete obstruction in femoral and SIE vessels (Fig. 2).

Changes 14 days after the injection of sclerotic agents

Fourteen days after the injection of sclerotic agents, we looked for changes in the tissue through the same incision. In all rats, we found that the subcutaneous tissues surrounding the vessels were fibrous; however, no obvious changes were observed in the femoral and SIE vessels themselves (Fig. 2). On pathological investigation, *en-bloc* necrotic nidus, which is usually found in avascular necrosis, was not observed in any of the samples. EO- and PL-injected samples showed enhanced inflammatory cell infiltration in loose areolar tissue, which enfolded vessels, and part of this cellular infiltration appeared to invade the outer membrane of vessels. These infiltrating changes were not obvious in the ET-injected samples. The integrity of the vascular lumen, endothelial cells and patency was maintained in all the samples (Fig. 3). Vascular patency was directly confirmed by injecting yellow-coloured silicone rubber through the proximal end of femoral vessels (Fig. 4).

Discussion

Intravascularly administered sclerosants are known to cause endothelial destruction and exposure of subendothelial collagen, resulting in activation of blood coagulation.[15] The mechanics for endothelial destruction are considered to be specific to each sclerosant, such as cellular fixation for ET, and cellular membrane disruption for surface activating agents such as EO and PL.[7]

In our previous experimental study, we evaluated the destructive ability of intravascularly administered sclerotic agents on surrounding tissues such as nerves[8] and skin[11] for the assessment of sclerotherapy-associated complications. Strong sclerotic agents

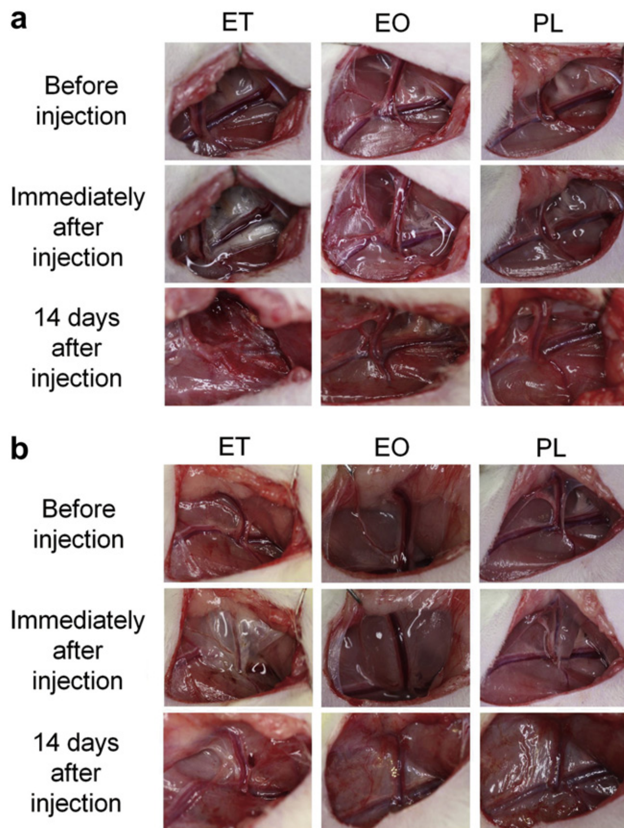

Figure 2. Representative findings of before, immediately after, and 14 days after the injection of sclerotic agents into surrounding tissue of femoral vessels (a) and superficial inferior epigastric vessels (b). No obstruction of vessels was observed with injection of absolute ethanol (ET), 5% ethanolamine oleate (EO) and 3% polidocanol (PL).

such as ET and EO showed the ability to damage surrounding tissues by leaking out of damaged vascular walls. Therefore, it is possible that extravascularly administered sclerotic agents are also able to destroy vessels. However, in our present study, we did not find any damage to vascular endothelial cells, as shown by injection of sclerotic agents into adjacent surrounding tissues, still less the obstruction of vessels even in tiny vessels in the rat groin fossa.

Two reasons could explain the different effects between intravascularly administered agents on surrounding tissues and extravascularly administered agents on endothelial cells. One reason could be structural differences in the administrated area. In our previous study,[8,11] because we used rat veins with partial or transient flow obstruction as an experimental model, the volume of the vascular lumen was relatively limited and, therefore, intravascular spaces attained comparatively high pressure with injection of agents, resulting in overflow of sclerotic agents outward. On the other hand, extravascular spaces in the rat groin fossa are composed of thin pliable tissues, and sclerotic agents are easily separated by thick and strong vascular walls. Second, the existence of vascular flow is the reason for differences. Since destructive effects of sclerotic agents on cells are correlated with the duration of contact,[7] continuously retained vascular current may improve washing out of sclerotic agents if extravascularly administrated agents reach the internal surface of the vascular wall (i.e., endothelial cells).

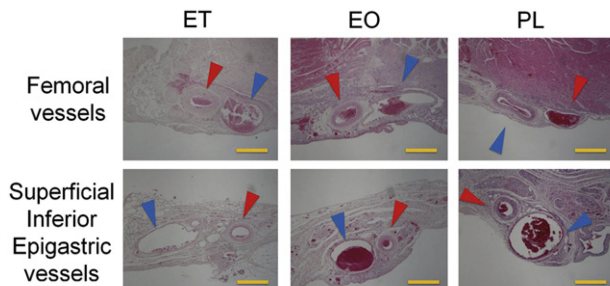

Figure 3. Representative findings of histology of femoral and SIE vessels. Fourteen days after injection of absolute ethanol (ET), 5% ethanolamine oleate (EO) and 3% polidocanol (PL), segments of vessels were obtained with surrounding tissues and stained with haematoxylin–eosin. The integrity of the vascular lumen is conserved. No obstruction of vessels is recognised. Red arrowheads indicate arteries and blue arrowheads indicate veins. The yellow scale bar indicates 500 μm.

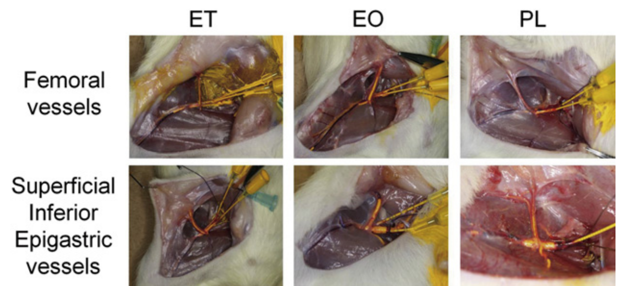

Figure 4. Representative findings of femoral and SIE vessels after silicon rubber injection. Using yellow-coloured silicon rubber, it was confirmed that there was no obstruction of vessels observed with injection of absolute ethanol (ET), 5% ethanolamine oleate (EO) and 3% polidocanol (PL).

In our experimental setting, extravascularly administrated sclerotic agents had little effect on vessels in the rat groin fossa. We consider that differences in structure and circulation dynamics of human AVMs and tiny vessels in the rat, such as a thicker vascular wall and higher vascular flow in human AVMs, enhance the resistivity to extravascularly administered sclerotic agents. Furthermore, increased capillary flow in AVM lesions may enhance clearance of interstitially deposited sclerotic agents. Therefore, no destructive influences are expected with extravascular administration of sclerotic agents.

On the other hand, extravascular interstitial tissues do not show *en-bloc* necrotic nidus formation with direct administration of sclerotic agents *in situ*. We consider that changes after sclerotic agents are different from typical tissue necrosis, such as those observed in avascular necrosis, because vascular structure is mostly preserved, although cellular necrosis and degeneration of the extracellular matrix occur to some degree. Further study is required to investigate this possibility in detail.

Based on our findings, we conclude that attenuation of vascular flow of arteriovenous shunts after extravascular injection of sclerotic injection observed on Doppler ultrasonography is transient and/or trivial, and does not cause disruption of vessels. In the treatment of AVMs, maximal effort should be made to deliver sclerotic agents into the vascular lumen to attain sufficient destruction of lesions and regulate blood flow.

Acknowledgement

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Conflict of Interest

The authors declare that they have no other competing financial interests.

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