Onyx in an experimental aneurysm model: histological and angiographic results

Laboratory investigation

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Object. The use of liquid embolic agents for endovascular treatment of cerebral aneurysms is evolving. The authors’ aim was to evaluate the use of Onyx HD-500 in an experimental aneurysm model and to obtain histological and angiographic long-term results.

Methods. Ten aneurysms were created using an elastase model in rabbits. The aneurysms were embolized using Onyx in combination with an inflated balloon. One animal died 1 day after embolization. The animals were divided into 2 different groups. The animals in the first group (4 rabbits) were killed at 3 months and those in the second group (5 rabbits) were killed at 6 months after embolization. A venous control angiogram was obtained, and the aneurysms were examined histologically.

Results. In both groups control angiograms demonstrated that all aneurysms were completely occluded. There were no signs of recanalization. Migration of Onyx was seen in 4 animals, leading to the death of 1. Histological examination not only proved the aneurysms to be occluded but also demonstrated a thin layer of endothelium at the neck of the aneurysm. The histological result was identical in both groups.

Conclusions. This is the first study reporting the formation of a neointima over the neck of aneurysms embolized with Onyx in a rabbit model. Although the technique is challenging and migration of the liquid embolic agent cannot always be prevented, Onyx has a great potential to achieve a durable occlusion of aneurysms.

Key Words • aneurysm model • liquid embolic material • Onyx embolization

Many different endovascular strategies are used for the treatment of aneurysms. In addition to surgical clipping, endovascular techniques (such as occlusion of the parent artery, coiling with Guglielmi detachable coils with or without auxiliary devices such as balloons or stents) are effective treatment options. Since publication of the International Subarachnoid Aneurysm Trial study,9 management of intracranial aneurysms presenting with subarachnoid hemorrhage has been changed in many centers, and neurointerventional strategies are accepted as alternative treatment options. However, the occlusion rate of coil-treated aneurysms is lower and the rebleeding rate seems to be slightly higher than surgically treated aneurysms, especially in wide-necked and fusiform aneurysms.9 Research for the best endovascular device to obliterate aneurysms is ongoing. Therefore it is necessary to evaluate new embolic materials in regard to their characteristics in the embolization of intracranial aneurysms and to determine the potential to occlude an aneurysm to ideally 100% in a stable condition. The injection of liquid embolic material is considered to be an efficient treatment, as injected liquid can totally fill the aneurysm lumen leaving no gap between the embolic material and the aneurysm wall, regardless of its shape. Onyx HD-500 (ev3) is a liquid biocompatible polymer dissolved in its organic solvent, DMSO. It consists of ethylene vinyl alcohol and tantalum powder added to the polymer/solvent mixture to obtain appropriate radiopacity. In combination with water, DMSO rapidly diffuses away from the mixture, causing in situ precipitation and solidification of the polymer. Onyx is widely used to embolize cerebral arteriovenous malformations, but some interventionalists have already used it in the treatment of intracranial aneurysms.13 The preliminary results have been published in the CAMEO trial10 and are encouraging. The use of Onyx is recognized as an alternative embolic technique, and seems to result in lower recanalization rates in large aneurysms. In this study we wanted to evaluate Onyx in an animal model of aneurysm occlusion. The goal was to
achieve angiographic long-term results including histomorphological evaluation of the treated aneurysms.

Methods

We chose an experimental aneurysm animal model as first described in 1999. Ten rabbits (body weight 3–4 kg) underwent a protocol approved by the animal research committee of our institution.

The aneurysms were created in the right CCA in New Zealand white rabbits as previously described. For aneurysm induction, the fur at the neck was shaved and the skin was disinfected. After induction of anesthesia, the right CCA was surgically exposed over a length of 3 cm. The distal part of the artery was permanently ligated. A 5 Fr sheath was introduced into the proximal lumen of the artery. A second ligature was fixed around the vessel with the sheath to fix and to tie the sheath to the artery. A conventional 3 Fr Fogarty balloon catheter was placed in the origin of the CCA in the brachiocephalic trunk under fluoroscopic control. A 1.9 F microcatheter was then placed parallel to the balloon through the sheath, and the tip of the catheter was placed on the balloon. The balloon was inflated with a mixture of 50/50 contrast material and saline (0.9%). In this situation the proximal CCA was isolated from the circulation. Porcine elastase (100 U) was injected via the microcatheter into the isolated artery. The incubation time was 20 minutes. After this procedure the balloon catheter and the microcatheter were removed. The sheath was removed, and the artery was ligated 2 cm above the brachiocephalic trunk to form the aneurysm sac. In this situation the proximal part of the CCA was exposed again to the circulation. The vessel wall was destroyed by elastase, and within 3–4 weeks an aneurysm began to evolve out of the proximal CCA. To complete the operation the fascias and the skin were closed with a running suture. The entire procedure lasted ~ 60–70 minutes and was easy to perform.

Three to 4 weeks after aneurysm induction, the right and left femoral artery were surgically exposed and distally ligated. A 3 and 5 Fr sheath were introduced into the right and left femoral arteries, respectively. Control series angiograms were obtained using a microcatheter (Tracker Excel 14, Boston Scientific/Target), which was placed with the aid of the 3 Fr sheath into the aortic arch to get an impression of the vessel anatomy and of the aneurysm. A microcatheter compatible with Onyx (Ultraflow 14, Micro Therapeutics, Inc.) was then placed into the center of the aneurysm using a 0.10 Transend wire (Boston Scientific/Target). A balloon catheter (HyperForm, Micro Therapeutics, Inc.) was advanced through the left femoral artery and placed across the neck of the aneurysm (Fig. 1A). The balloon was inflated with a mixture of 50/50 contrast material and 0.9% saline. The diameter of the balloon was flexible and was adapted to the individual situation. A control angiogram obtained after 0.5 ml contrast material was injected rapidly through the Ultraflow microcatheter confirmed the occlusion of the aneurysm’s neck through the balloon (Fig. 1B). The microcatheter was flushed with 0.3 ml of DMSO within a period of ~ 120 seconds to displace the water from the microcatheter. Using a roadmap, the Onyx was injected slowly into the aneurysm until satisfactory embolization was performed. It was not possible to obtain control angiograms during the intervention. We injected Onyx at a rate of 0.1–0.2 ml per minute, and the injection time ranged between 4 and 8 minutes. The balloon was not deflated during the injection period in any case. We aimed for complete occlusion of the aneurysm as visible on the road map. If migration of Onyx was detected, the injection was terminated immediately and was not continued. Three to 4 minutes after termination of the Onyx injection, the balloon and the microcatheter were removed. A new microcatheter (Tracker Excel 14, Boston Scientific/Target) was again advanced into the aortic arch to obtain a control angiogram (Fig. 1C). Preembolization and postembolization angiograms were examined to evaluate the anatomic results (occlusion rate) and migration/protrusion of Onyx into the parent artery.

The animals were divided into 2 groups of 5 animals each. Three months after embolization a control angiogram was obtained in 4 rabbits (1 animal died 1 day after embolization), and the animals were killed. The remaining 5 rabbits underwent control angiography 6 months after embolization, and then the aneurysms were explanted for histomorphological evaluation.

In both groups 1000 IU heparin was administered intravenously before the embolization procedure to diminish thromboembolic complications. After the procedure the animals were treated with aspirin, which was dissolved in drinking water. Follow-up angiograms were obtained using a venous route (ear vein), with injection of 4 ml contrast material followed by a saline flush of 5 ml, as an arterial approach was not possible because of the previous ligation of both femoral arteries (Fig. 1D). After thoracotomy, the aortic arch and the proximal great vessels with the aneurysm were exposed and dissected from the surrounding tissue (see Fig. 3A). The samples were fixed in 4% neutral buffered formalin at room temperature and were embedded into paraffin to facilitate sectioning. The samples were stained using H & E and van Gieson. All sections were prepared, viewed, and analyzed by an experienced neuropathologist.

Results

Aneurysms were induced in all animals, and the diameter of the neck was between 3 and 5 mm. All animals tolerated the induction well. Technically, all aneurysms were successfully embolized. The initial occlusion rate directly after the aneurysm embolization procedure was 100%. One animal died 1 day after the embolization procedure due to parent vessel occlusion (brachiocephalic trunk) by migrating Onyx. Therefore 9 animals remained in the study: 4 animals were killed at 3 months and the remaining 5 animals at 6 months. The angiographic findings in both groups showed a completely occluded aneurysm. Recanalization or regrowth was not observed. There was no difference between the animals killed at 3 and 6 months with regard to the immediate and long-term angiographic results.

In 3 rabbits, a small amount of Onyx migrated along the microcatheter to the proximal brachiocephalic trunk, but not distally into the subclavian artery (Fig. 2A). The Onyx adhered to the vessel wall of the brachiocephalic trunk. These animals (2 were from the group of animals killed at 6 months, 1 was from the group of animals killed at 3 months) survived the intervention without obvious prob-
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Fig. 1. Angiograms demonstrating the procedure.  
A: The microcatheter is placed in the aneurysm and the balloon is positioned over the neck. The contrast agent is injected through the microcatheter.  
B: The balloon is inflated and, using a road map, Onyx is injected.  
C: After injection a new microcatheter is placed in the brachiocephalic trunk and the aneurysm is completely occluded.  
D: Image obtained at 6 months through an ear vein, showing a completely occluded aneurysm in stable condition.

Fig. 2. Angiograms showing complications during embolization.  
Left: The Onyx migrated proximal to the origin of the left CCA (arrow). The animal survived without any problems.  
Right: The catheter is stuck in the cast. A second catheter was advanced in the origin of the brachiocephalic trunk to obtain the image.
lems. In another case, the microcatheter was stuck in the Onyx cast and could not be removed (Fig. 2B). The catheter was subsequently cut at the level of the femoral artery and remained in an intravascular location. This animal was killed at 6 months, as planned.

Histological evaluation showed that the walls of the aneurysms were thinner than those of the aneurysm-bearing vessels. The elastic lamina had been dissolved as intended. No inflammation or foreign body reaction was observed in the adjacent tissue of the aneurysm. All aneurysms were completely occluded, and there were no signs of recanalization (Fig. 3B). We found a thin layer of endothelium over the entire neck of all aneurysms (Fig. 3C). With regard to the histomorphological results there was no difference between the animals killed at either time period.

Discussion

Endovascular strategies are more and more accepted as alternative treatment options in patients with subarachnoid hemorrhage caused by aneurysm rupture. Coils provide a safe and effective treatment for many aneurysms. However, wide-necked and large or giant aneurysms are still a challenge. When coils alone are used, a permanent occlusion in these cases is difficult to achieve. The use of liquid embolic agents is a possible alternative for treatment of some aneurysms. However, experience with this challenging technique is limited. Only a few centers contributed to the largest trial, the CAMEO study; 100 aneurysms were treated at 20 European centers. Of these aneurysms, 38 were treated in Ankara, Turkey, and 7 in Istanbul, Turkey. Therefore, the other centers treated an average of 3 patients. It is obvious that embolization with Onyx is not a standard procedure like coiling. Histomorphological data and animal model data are rare.

Acar et al. used a sidewall model in New Zealand white rabbits and created 5 aneurysms. These authors measured the intradomal aneurysm pressure and found a decrease in...
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pressure even in incompletely occluded aneurysms. Histomorphological evaluation of the aneurysms was not performed.

Murayama et al. investigated the combination of Onyx and balloon protection in a sidewall porcine aneurysm model. They measured the IAP and 7 aneurysms were investigated. They found that the IAP increased during the injection of Onyx. To reduce a sudden increase in pressure, the authors undertook subtotal occlusion of the neck. After 90% occlusion of the aneurysm with Onyx they performed complete occlusion of the parent vessel with the balloon and subsequently complete filling of the remaining space of the aneurysm. Despite this careful technique the authors also noted migration of Onyx in 2 of 7 cases. They concluded that even full inflation of the balloon does not always seal the aneurysm neck completely. The elevated intraneurysmal pressure may extend the small, tight space between the balloon and parent vessel wall. However, no critical histomorphological examination was performed, probably due to the fact that histomorphological results in a surgically created sidewall aneurysm have little impact, as postoperative changes might predominate.

For this reason we used an elastase model in rabbits, thus avoiding any postoperative changes at the neck of the aneurysm. Local surgery should be avoided to minimize a healing response that might impair the histomorphological interpretation of the reactions to the embolic agent. The rabbit model is widely accepted to represent human intracranial aneurysms as the fibrinolytic system of the rabbit mimics that of humans. The physical dimensions and radiographic appearance are comparable to human aneurysms. Also, the hemodynamic forces in the aneurysm are similar to those in human bifurcation aneurysms. Previous studies have shown that the wall, of these aneurysms lack an elastic lamina, just as in human aneurysms. Also, the vessel anatomy of the aortic arch in New Zealand white rabbits is the same as that in humans. Some limitations of the model are obvious. The aneurysms are created in the mediastinum and not within the subarachnoid space. The aneurysm’s environment is therefore different from that of an intracranial aneurysm. Generally, in 10% of induced aneurysms, the aneurysms will occlude after their induction, but this was not observed in our series. Despite all limitations the model is accepted as the best model to simulate intracranial aneurysms.

We found the occlusion of the dome and main part of the aneurysm easy to perform; however, occlusion of the neck of the aneurysm closest to the balloon was technically challenging to avoid migration of Onyx. To avoid high IAP we injected Onyx slowly, as the blood needs a pathway out of the aneurysm during injection and filling. Thus migration of Onyx into the parent vessel is difficult to avoid and occurred in 4 cases in our study, resulting in the death of 1 animal. We believe that the balloon does not seal the aneurysm in the portion covering the microcatheter as the balloon might not wrap the catheter completely. This could cause migration of Onyx along the microcatheter and not in the distal portion, where the balloon has direct contact with the surface of the vessel.

Earlier studies comparing the histomorphological occlusion rate of aneurysms under different anticoagulation regimens in which Guglielmi detachable coils were used reported a mean occlusion rate of only 91%. In an experimental study Reul et al. stated that angiographically completely occluded aneurysms in rabbits showed signs of recanalization in the dome of the aneurysm in the histomorphological evaluation. We achieved 100% occlusion in all our Onyx-treated aneurysms. Histomorphological evaluation showed no recanalization of the aneurysms. We found a thin layer of neointima at the neck of all Onyx aneurysms (Fig. 3). To our knowledge this is the first report of endothelialization with neointima covering the aneurysm neck in experimental aneurysms occluded with Onyx.

The technique is challenging and has some intrinsic limitations. One limitation is the delivery of embolic material without the possibility of repositioning or retrieval. Migration has to be controlled by balloon inflation across the neck of the aneurysm. Another device-related complication is fixation of the microcatheter in the Onyx cast. We think that our relatively high complication rate is part of a learning curve as well as device related. There have been no reports on stuck catheters during embolization of aneurysms with Onyx; even in the CAMEO trial, there is no mention of catheters that have become stuck. Nonetheless Weber et al. reported a 4% rate of stuck catheters during arteriovenous malformation embolization without clinical consequences.

Another unsolved problem is the occlusion time. In an experimental model in rabbits an occlusion time of > 5 minutes without deflating the balloon may be acceptable, but not in humans. As we had only 10 animals in our series, a larger population is necessary to confirm our results.

Conclusions

This is the first study that showed neointima covering the neck of an aneurysm embolized with Onyx. This liquid embolic agent has been shown to have the potential to occlude aneurysms in a stable and durable condition, but further investigation is necessary given that the technique is challenging.

Disclaimer

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References

6. Grunwald IQ, Romeike B, Eymann R, Roch C, Struffert T, Reith...


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