Development of the stent capable for controlled release of basic fibroblast growth factor and argatroban to treat cerebral aneurysm –In vitro experiment and evaluation in a rabbit aneurysm model

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An ideal stent for treating cerebral aneurysms should have an antithrombotic effect on the inner stent blood-facing side and a tissue organizing effect on the aneurysmal side. A new stent graft to treat cerebral aneurysms was designed by combining dual release of organization-promoting and antithrombotic drugs. Some growth factors, basic fibroblast growth factor (bFGF), transforming growth factor, and vascular endothelial growth factor, have been shown to promote fibrous formation and endothelialization in an in vivo study.[8-10]In fact, bFGF is an ideal factor for promoting aneurysm organization. It has been reported that the sustained release of bFGF from gelatin hydrogel-coated coils accelerates fibrous formation in the aneurysm cavity.[10-12]Based on this, the stent was coated with a gelatin hydrogel incorporating bFGF for sustained release of bFGF. In addition, PLGA microspheres containing argatroban, an antithrombin drug, were prepared to coat the stent by mixing them with the gelatin hydrogel. The objective of this study was to design a stent capable of dual and bidirectional release of bFGF and argatroban and evaluate its therapeutic efficacy in a rabbit aneurysm model.

Method

Argatroban of an anti-thrombotic drug was encapsulated in biodegradable poly (D,L-lactide-co-glycolide)(PLGA) microspheres for the controlled release. The in vitro study for the release test and anticoagulation behavior of released drug was performed.

Basic fibroblast growth factor (bFGF) of an organization drug was released from gelatin hydrogels. Stents were coated with gelatin hydrogels mixing PLGA microspheres containing argatroban and those containing bFGF. Briefly, we purchased bare metal, balloon-expandable stents (Integrity: diameter, 3.5 mm; length, 12 mm; Medtronic, Minneapolis, Minnesota). These stents were taken out from the balloon. For the inner coating, the stent mounted on a plastic mold (diameter 3.5 mm) was coated with 50 μl of 5 wt% gelatin aqueous solution mixed with 10 μl of 25 wt% glutaraldehyde solution dispensing 5 mg of PLGA microspheres containing argatroban, followed by leaving it at 4°C for 12 h for gelatin crosslinking. Then, the resulting stent was immersed in 100 mmol/l of glycine aqueous solution, agitated at 37°C for 15 min to block the residual aldehyde, and washed three times with DDW. The gelatin-coated stent was then freeze-dried. For the outer coating, the freeze-dried stent mounted on a plastic mold was dipped into the same gelatin aqueous solution mixed with glutaraldehyde, followed by crosslinking of gelatin at 4°C for 12 h. Then, the stent was similarly washed with the glycine solution and DDW, freeze-dried, and sterilized with ethylene oxide gas. To impregnate bFGF into the gelatin hydrogel coating, 10 μl of 100 μg of human recombinant bFGF solution (Kaken Pharmaceutical Co., Ltd., Tokyo, Japan) were homogenously dropped onto the gelatin hydrogel and maintained at 4°C overnight to allow bFGF to sorb into the gelatin hydrogel coating.[15] Final stents were crimped on balloon catheter. We crimped slowly and kindly on circumference because roughly

treating might make a coating cracked. As a control, a similar coating of gelatin hydrogel was prepared without any drugs. The gelatin-coated stents for the control study were prepared by performing the above process without drugs. and applied to the aneurysm neck of elastase-induced rabbit model. The follow-up DSA was performed at 1 or 2 weeks after the endovascular treatment. In-stent thrombus was classified based on the TIMI thrombus grade[17] For histological evaluation, aneurysms were excised from the parent artery, cut longitudinally in the midpoint, and embedded in paraffin. Then, 5-μm-thick slices from cut sections were obtained and stained with Masson-trichrome to view collagen fibers. Immunostaining with a mouse monoclonal anti-vimentin antibody (Abcam,plc., Cambridge, Japan) was also performed to identify fibroblasts. The area of the non-thrombotic site in the aneurysm was assessed by Image J software (National Institutes of Health, Bethesda, MD, USA).

Result

Characterization of PLGA microspheres containing argatroban

About 40% Argatroban of the initial loading was gradually released with time from PLGA microspheres containing argatroban over about 2 weeks, but, thereafter, no substantial release was observed. Although the release rate seemed to be slightly fast, argatroban was also released from PLGA microspheres containing argatroban incorporated in the gelatin hydrogel.

Animal experiments

Complete obliteration was obtained in 3 of 6 rabbits with the stent incorporating bFGF and PLGA microspheres containing argatroban and a neck remnant was obtained in 1 of 6 rabbits treated with the stent incorporating bFGF and PLGA microspheres containing argatroban. On the other hand, no complete obliteration was observed in the drug-free stent group. In–stent occlusion was seen in one of six rabbits treated with the drug-free stent. The TIMI thrombus grade was significantly different between the two groups. ($p=0.0395$)

Most of aneurysm cavity was occupied by loose connective tissues for the group treated with stents incorporating bFGF and PLGA microspheres containing argatroban whereas extensive massive hematoma was observed for the drug-free stents.

In the group treated with stents incorporating bFGF and PLGA microspheres containing argatroban, the percentage of area occupied by non-thrombotic tissue after one week was 81.2%±5.3%, significantly larger than that with drug-free stents (52.6%±5.4%; $p<0.0193$), and the percentage of area occupied by non-thrombotic tissue after two weeks was 82.7%±10.4%, significantly larger than that of drug-free stents (48.7%±18.4%; $p<0.0248$).

DISCUSSION

It is known that bFGF stimulates the proliferation and migration of endothelial cells, smooth muscle cells, and fibroblasts, as well as the production of Type 1 collagen.[21-23] Based on this, it is an ideal growth factor for promoting organization in an aneurysm. However, its in vivo half-life is very short, and its effects are not always seen when free bFGF is used. In this study, the acidic gelatin hydrogel was prepared by chemical crosslinking for the sustained release of bFGF; bFGF is released from the gelatin hydrogel when the hydrogel is enzymatically degraded in vivo.[14]

Argatroban is an agent that directly inhibits thrombin, thereby competitively inhibiting fibrinogen cleavage and platelet activation stimulated by thrombin.[24] Unlike heparin acting indirectly on thrombin, it is characterized by fewer differences in efficacy among individuals. Therefore we used argatroban as an antithrombotic drug, and aimed to developed gelatin coated stents incorporating bFGF and argatroban.

In the present study, most of the aneurysm cavities were occupied by loose connective tissue in the group with stents incorporating bFGF and PLGA microspheres containing argatroban. Compared with the drug-free stent group, the non-thrombotic area was significantly broader in the group with stents incorporating bFGF and PLGA microspheres containing argatroban. Taken together, the pathological examination indicated a process of aneurysm organization. It is possible that the stents incorporating bFGF and PLGA microspheres containing argatroban accelerate the organization process within the aneurysm.

There have been no studies to confirm the locally effective amount of argatroban that is required to prevent in-stent thrombosis. While the amount of argatroban released from the present stent might be small[26], but we did not calculate the amount of argatroban released from PLGA microspheres containing argatroban in vivo. Although we demonstrated that approximately 40% of the initially loaded argatroban was released from the PLGA microspheres during the in vitro study, the overall recovery rate for final stent product was unclear. Therefore, the amount of released argatroban during the in vivo study might be less than in vitro study. However, it is conceivable that the present drug-coated stent would be useful for preventing in-stent thrombosis based on the results of our experiments.

Conclusion

The stent incorporating bFGF and PLGA microspheres containing argatroban is an effective device for cerebral aneurysm treatment.

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