Prevention of Carotid Artery Restenosis After Sirolimus-Coated Stent Implantation in Pigs

Gunnar Tepe, MD; Peter Muschick, PhD; Michael Laule, MD; Frank Reddig, MD; Claus D. Claussen, MD; Ludger M. Dinkelborg, PhD; Hans Tielemans; Manfred Wehrmann, MD; Stephan H. Duda, MD

Background and Purpose—To test the feasibility of self-expanding drug-coated nitinol stents for prevention of restenosis in an animal model. Stent implantation in the carotid artery (CA) has been shown to be feasible for treatment of CA stenosis. Even though the restenosis rate in CA is reported to be lower than in the coronary and peripheral arteries, problems may arise with increasing numbers of treated patients and lengthier follow-up.

Methods—After predilatation with 8-mm balloons, 8 Goettinger minipigs were randomly selected to receive a sirolimus-eluting self-expanding nitinol stent (7 mm/80 mm) as well as the same stent without sirolimus/polymer coating in the right or left CA. Aspirin was given starting 3 days before the intervention and administered for an additional 4 weeks. Clopidogrel was administered for 10 days.

Results—After 6 weeks, 2 subacute occlusions were observed in both groups. In the remaining vessels, the neointima was significantly reduced by sirolimus/polymer-coated stents (5.9 ± 2.5 versus 0.7 ± 1.0 mm²).

Conclusions—Sirolimus self-expanding nitinol stents may be an effective tool in reducing neointimal formation in CA.

Key Words: atherosclerosis • carotid arteries • stents

Silent implantation in the carotid artery (CA) has been shown to be safe and effective and seems to be a feasible, minimally invasive alternative for high-grade symptomatic and asymptomatic stenosis of the internal CA (ICA). However, with increasing numbers performed and lengthier follow-up, in-stent restenosis (stenosis >50%) might occur more frequently in CA.1-3 Drug-eluting stents have been shown to be a powerful tool in reducing in-stent restenosis. They have already been approved for coronary application and are currently under clinical investigation for the superficial femoral artery and the renal artery.4-7 This is the first report of sirolimus-eluting stents for prevention of in-stent restenosis in CAs of pigs.

Materials and Methods

All experimental procedures were approved by the regional governmental authorities and complied with the American Heart Association guidelines for use of animals in research. Eight male Goettinger minipigs, average age 1.2 years and weighing 29 to 35 kg, were used in this study. The animals were randomly selected to receive a sirolimus-eluting self-expanding nitinol stent as well as the same stent without sirolimus/polymer coating (SMART stent; Cordis; n=16) in the right or left CA. All procedures were performed blinded to the stent characteristics.

Animal Model

All procedures were performed under general anesthesia, and all animals received standard therapy for periprocedural infection control. An 8F sheath was placed in the right common femoral artery under ultrasound guidance. For balloon injury, the common CA was predilatated with 1 balloon inflation with a diameter of 8 mm at 10 atm for 30 seconds (total length of 8 cm). Based on an average CA diameter of 6 mm in pigs of this size, the balloon/artery ratio was 1.3/1. The 7 mm/80 mm self-expandable SMART stents were placed in the same area where predilatation had been performed and were postdilatated with 6-mm balloon. Each pig was treated with oral aspirin (500 mg per day) starting 3 days before the procedure and continuing 4 weeks after intervention, and with clopidogrel starting with a loading dose (300 mg) 1 day before stent implantation followed by daily administration of 75 mg per day for 10 days. In addition, intravenous heparin (150 to 200 U/kg) was given directly after sheath insertion and then twice daily until day 2.

Follow-Up

After 6 weeks, angiographic and intravascular ultrasound (IVUS) controls were performed. The procedures were performed under general anesthesia as described above. Calculation of the degree of

Received March 25, 2005; final revision received June 20, 2005; accepted June 22, 2005.

From the Departments of Diagnostic Radiology (G.T., F.R., C.D.C., S.H.D.) and Pathology (M.W.), University of Tuebingen, Germany; the Schering Company (P.M., L.M.D.), Berlin, Germany; the Department of Internal Medicine (M.L.), Charité, Berlin, Germany; and Cordis (H.T.), a Johnson & Johnson Company, Warren, NJ, and Waterloo, Belgium.

Peter Muschick and Ludgar Dinkelborg are employees of Schering AG, Berlin Germany. Hans Tielemans is an employee of Cordis, a Johnson & Johnson Company, Warren, NJ, and Waterloo, Belgium.

Correspondence to Gunnar Tepe, Department of Diagnostic Radiology, University of Tuebingen, Hoppe-Seyler-Str. 3, D-72076 Tuebingen, Germany. E-mail gunnar.tepe@med.uni-tuebingen.de

© 2006 American Heart Association, Inc.

Stroke is available at http://www.strokeaha.org

DOI: 10.1161/01.STR.0000199080.94177.ba

492
stenosis and the neointimal formation were performed with a computer-assisted program analyzing the IVUS data.

**Statistical Analysis**
Comparison between the 2 treatment groups was made with Mann–Whitney-Wilxon and $t$ tests. Values are expressed as mean $\pm SD$. A value of $P<0.05$ was considered statistically significant.

**Results**
All stents were successfully implanted. Immediately after stent placement, there was no evidence of procedure-related dissection or improper stent inflation or placement. No animal died prematurely. After 6 weeks, 4 of 16 CAs were totally occluded. One animal presented with occlusion of both CAs, but because of vertebral artery supply, no symptoms were observed. One CA was occluded in 2 other animals. None of the animals experienced a stroke. Two sirolimus-eluting stents and 2 control stents were found with no blood flow. All 4 stents were completely thrombosed with no residual lumen. Therefore, an IVUS could not be performed on these animals. The animal with total occlusion of both CAs was euthanized after 6 weeks. The histology showed an old thrombus formation (organized by fibrous tissue) with only a thin or no neointima. In the remaining vessels, the neointimal area measured by IVUS was $0.7 \pm 1.0 \text{ mm}^2$ in the sirolimus-eluting stent group and $5.9 \pm 2.5 \text{ mm}^2$ in the control stents. This difference was significant ($P=0.004$). The data are illustrated in the Figure. No enhanced neointimal buildup was observed either at the stent edges or in the proximal or distal stent segment. The in-stent restenosis pattern did not follow any rules.

**Discussion**
Together with protection devices against embolization during ICA stenting, at least in patients with coexistent comorbidities, CA stenting appears to be associated with a lower rate of periprocedural complications. The reported results of the SAPPHIRE trial of stenting versus endarterectomy documented stroke, myocardial infarction, or death in 12.6% at 30 days in subjects randomized to endarterectomy compared with only 5.8% in those randomized to stenting. Although essential questions regarding the effectiveness of CA stenting are yet to be answered, there is interest in defining other determinants of clinical success. Restenosis is such a secondary outcome. However, the ICA supplies neurological territory, and, unlike in coronary or in the superficial femoral artery, ischemic symptoms appear to develop more often from embolization than from flow reduction. Indeed, a restenotic lesion that consists of many smooth muscle cells may behave differently from that of the primary atherosclerotic one. Nevertheless, as the first long-term data of that new technology became available, 1-year restenosis rates (defined as stenosis of $\geq 50\%$) between 8% and 22% were reported. It should be noted that the quoted series were small and performed with a previous generation of stents. In well-performed prospective studies such as the SAPPHIRE trial, restenosis rates were significant at $<5\%$. The risk of restenosis is very low after CAS, although it is not 0, and there may be a need for drug-eluting stents (DESs), particularly in patients with a history of radiation therapy or

The risk of restenosis is very low after CAS, although it is not 0, and there may be a need for drug-eluting stents (DESs), particularly in patients with a history of radiation therapy or in-stent stenosis because treatment options in those individuals are limited.

Interestingly, Schillinger et al reported that the increase of the C-reactive protein 48 hours after CA stent implantation can be used to predict 6-month restenosis. The importance of inflammation during ICA stenting was also supported by a report of Toma et al, who described focal persistence of chronic inflammation with neointimal formation with mural thrombus formation in a human CA after stent implantation. Inflammation and neointimal formation are both targeted by sirolimus-eluting stents.

As far as the investigators are aware, no animal data on self-expanding DESs in CAs exist to date. Sirolimus-eluting nitinol stents are completely different from the stainless-steel balloon-expandable CYPHER stents, which have been investigated in clinical trials. Besides the stent itself, the polymer coating, which may have an impact on the clinical outcome by an influence on thrombogenicity and inflammation, was different from the polymer coating used on CYPHER stents.
In addition, the release kinetics varied from the CYPHER stents used in the coronaries. In the present study, stents with characteristics similar to those used in the Siroccot trial (sirolimus-coated SMART nitinol stents for application in the superficial femoral artery) were used.6

The high rate of subacute occlusions is most likely related to an inadequate peri-interventional and postinterventional platelet inhibition. Aspirin was continued for 4 weeks, and clopidogrel was stopped 1 week after stent implantation. In reviewing the animal data, there are only a few studies with stent implantation in the CA. After balloon angioplasty only, pigs received either heparin with no further antiplatelet therapy18 or aspirin for 4 weeks.16,17 Under these treatment modalities, no subacute occlusions were observed. Nevertheless, Bienvene et al18 described a relationship between platelet adhesion and the degree of injury (single or repeat; mild or deep) induced by angioplasty of CA in pigs. Our model with overstretch angioplasty followed by stent administration is a double-injury model, which is most likely accompanied by enhanced platelet activation. In addition, the lesion length with 8 cm in total exceeded those tested in animal models or in clinical situation, which are normally limited to a maximum of 4 cm. With ≥30 days of clopidogrel, an extremely low acute–subacute stent thrombosis has been reported in clinical trials.2,10,19 Therefore, subacute CAS thrombosis is no problem in clinical practice. With DESs, a need for prolonged treatment with clopidogrel is well accepted.5,7,15 Currently, there are no data that support the influence of clopidogrel on neointimal formation and restenosis.

Study Limitations

The study is limited because of high incidence of thrombosis in both groups, most likely caused by the insufficient platelet therapy and the trauma attributable to overstretch angioplasty. In addition, it has to take in account that animal models might not be parallel with the human clinical situation.

Summary

This study shows that sirolimus-eluting stents may be as effective in the CA as in the coronary arteries. Whereas in the control group, clear neointimal thickening was observed, the neointima was almost absent in the drug-eluting stent group. Sirolimus-eluting stents were not accompanied by a higher degree of thrombotic events despite a suboptimal antiplatelet therapy. This may be interpreted as a sign of adequate balance between inhibition of cell proliferation and endothelial recovery. Animal models of restenosis are generally performed for 4 to 6 weeks, simulating the clinical situation after 6 months. Of course, long-term effects have to be questioned. Therefore, this study will be performed again in 6 months to investigate the long-term effect of sirolimus-eluting stents in CA of pigs.

References

Prevention of Carotid Artery Restenosis After Sirolimus-Coated Stent Implantation in Pigs

Gunnar Tepe, Peter Muschick, Michael Laule, Frank Reddig, Claus D. Claussen, Ludger M. Dinkelborg, Hans Tielemans, Manfred Wehrmann and Stephan H. Duda

Stroke. 2006;37:492-494; originally published online December 29, 2005;
doi: 10.1161/01.STR.0000199080.94177.ba
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2005 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/37/2/492

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org/subscriptions/