

Safety and biocompatibility of a novel self-expanding nitinol carotid stent with hybrid cell design in a porcine model of neointimal hyperplasia

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Abstract

Background: Stent design may influence the outcomes, suggesting that adverse event rates vary according to free cell area and cell design. Open cell design technology of self-expandable stents, dedicated for carotid revascularisation has better deliverability, although closed cell technology is expected to cause fewer thromboembolic events.

Aim: To evaluate the feasibility and vascular response of novel, hybrid cell, self-expandable nitinol stents (MER[®], Balton, Poland) implanted into porcine carotid arteries. Hybrid cell design combines open and closed cell technology.

Methods: All tested stents were implanted with 10% overstretch into 10 carotid segments of Polish domestic pigs. Control angiography was obtained immediately before and after vascular interventions as well as 28 days after the procedure. Thereafter, animals were sacrificed, and the treated segments were harvested and evaluated in the independent histopathology laboratory.

Results: All stents were easily introduced and implanted, showing good angiographic acute outcome. At 28 days, in the angiography, all vessels were patent with no signs of thrombi or excessive neointimal formation, with the late lumen loss of -0.11 ± 0.3 mm and percentage diameter stenosis $10.18 \pm 8.1\%$. There was a 10% increase in the vessel reference diameter when compared to baseline (4.57 ± 0.5 vs. 4.96 ± 0.3 mm, $p < 0.01$). In the histopathology, mean area stenosis was 17.4% and mean intimal thickness was 0.20 mm. At histopathology, the mean injury, inflammation, and fibrin scores were low. Endothelialisation was complete in all stents, and neointimal tissue appeared moderately mature as shown by the moderate mean neointimal smooth muscle score. Nonetheless, histopathology shows one stent affected by peri-strut granulomas and one stent affected by marked mineralisation.

Conclusions: The novel Polish self-expandable nitinol carotid stent with hybrid cell technology shows optimal biocompatibility and a vascular healing profile, and therefore may be introduced for first-in-man application.

Key words: carotid stenting, new technologies, pre-clinical study

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INTRODUCTION

Carotid artery stenosis is the most common cause of stroke and subsequent disabilities in developed countries. Although carotid endarterectomy (CEA) remains the gold standard for its

treatment, carotid artery stenting (CAS) has been widely utilised as an alternative approach, especially in situations when CEA has been contraindicated due to severe comorbidities or unfavourable anatomy [1–3]. In order to further improve the

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outcomes of CAS and to expand clinical indications for this technique further research and technology improvements are required. Recent studies have shown that stent design could influence the outcomes, suggesting that adverse event rates vary according to free cell area and cell design [4, 5]. Therefore, the aim of this study was to assess safety, feasibility of implantation, and vascular response of a novel self-expandable nitinol MER® stent (Balton, Warsaw) with hybrid cell technology implanted into porcine carotid arteries.

METHODS

Device description

The studied device is a self-expandable nitinol stent with tantalum markers on the ends. The stent is cut off from the nitinol tube by a laser. It has hybrid cell technology, which combines repeating unicellular modules comprised of “z-like” shaped cells (Fig. 1) throughout the length of the stent. The mean cell area is 7.6 mm². The range of cell areas is 6.2–9.3 mm². The delivery system is monorail and 5 F compatible. All devices were 6 × 20 mm.

Experiment design and procedure

The experiment was performed in the Centre for Cardiovascular Research and Development (CCRD) of American Heart of Poland. The study protocol was approved by the local ethics committee for animal research. A total of five animals received standard care outlined in the study protocol and in accordance with the act of animal welfare and the “Principles of Care of Laboratory Animals”.

Three days prior to the procedure, dual antiplatelet therapy consisting of 75 mg of clopidogrel and aspirin per day was initiated and continued until the termination. All pigs were fasted overnight before stent implant procedure. Animals were pre-medicated with atropine (0.5 mg) and subsequently sedated with intramuscular ketamine hydrochloride (20 mg/kg) and xylazine (2 mg/kg), intubated, and anaesthetised with an intravenous propofol bolus (20–40 mg) followed by a continuous infusion (2–4 mg/kg/h). Electrocardiogram and blood pressure were continuously monitored. A vascular sheath (6 F) was placed in the right or left femoral artery utilising Seldinger technique. Anticoagulation with heparin was achieved (3,000–10,000 U) to maintain a coagulation time ≥ 250 s. Following angiography, all vessels were sized for proper stent implantation after live quantitative vascular

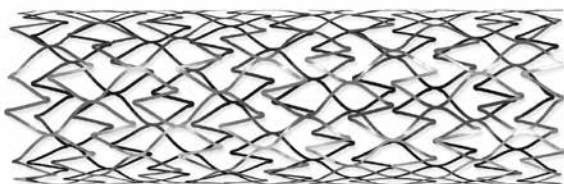


Figure 1. Hybrid cells self-expanding carotid

angiography (QVA) analysis. Subsequently, two stents were implanted, one in each carotid artery.

All pigs were prepared, anaesthetised, and catheterised in the same fashion as described above after 28 days. Following control angiography, the treated segments were carefully harvested, flushed, and stored in formalin and sent to the independent pathology laboratory for analysis (AccellAB Inc., Boisbriand, Quebec, Canada).

Quantitative vascular angiography

Carotid angiographies were obtained using the Siemens Coroskop Millennium Edition angiographic unit (Siemens AG, Munich, Germany). A Multipurpose 6 Fr guiding catheter was utilised for angiography and stent implantation. QVA analysis was performed in a blinded fashion utilising QAngio XA Software version 7.1.14.0 (Medis Medical Imaging Systems, Leiden, The Netherlands) from two contralateral projections. The baseline and 28-day follow-up reference vessel diameters were taken from the proximal and distal portions of the treated segments using the guiding catheter as a standard for measurement. The balloon-to-artery ratio was calculated. Percentage diameter stenosis (%DS) at follow-up was calculated as: $[1 - (\text{minimal lumen diameter}/\text{reference vessel diameter})] \times 100\%$.

Histology and histomorphometry

The stented segment of each artery was embedded in methyl methacrylate. Three blocks — proximal, medial, and distal — were cut from each artery. From these blocks two sections were grinded and polished to the final thickness of 60 μm or less. One section was stained with Verhoeff-Van Giesson [VVG] for elastin, and the other with haematoxylin and eosin (H & E). VVG-stained samples were digitalised, and then histomorphometry measurements were taken by Image Pro Plus software. The parameters evaluated were external elastic lamina area (EEL), internal elastic lamina area (IEL), luminal area (area bounded by the luminal border), medial area (EEL area – IEL area), intimal area (IEL area – luminal area), area stenosis $[1 - (\text{luminal area}/\text{IEL area}) \times 100]$, and mean intimal thickness (average of the distances between the IEL and the luminal border tracings, provided by the software). All H & E- and VVG-stained artery sections were examined by the study pathologist for semi-quantitative and descriptive histopathology. Five scores were assigned to each stented section using the criteria published previously [6]. Each strut in the section was scored, and means from each section were calculated and reported (Fig. 2).

Statistical analysis

The parametric variables are presented as mean ± standard deviation. Student’s paired t-test was used to compare the baseline vs. follow-up angiographic data. A value of $p \leq 0.05$ was considered statistically significant. GraphPad Prism 5® was used for analysis.

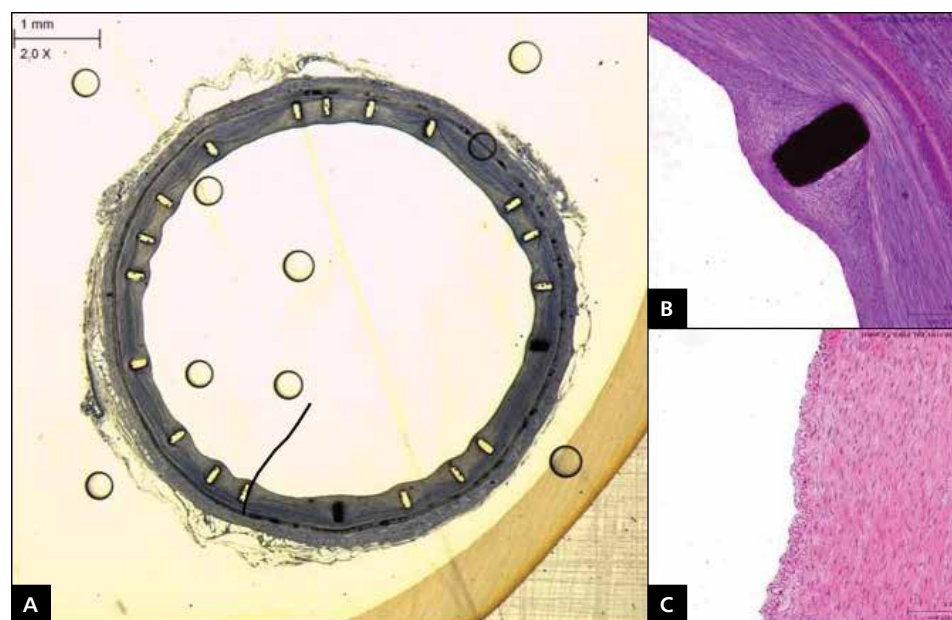


Figure 2. Representative microscopic section of carotid artery 28 days after MER® stent implantation; $\times 2$, VVG (A). Stent strut compressing media, internal elastic lamina layer intact (injury 0); $\times 20$, H & E (B). Normal, mature and endothelialised neointima; $\times 20$, H & E (C)

Table 1. Quantitative vascular angiography

	Pre-implantation (IQR)	Follow-up at 28 days (IRQ)	P
Minimal lumen diameter	4.4 (0.57)	4.51 (0.3)	0.089
Reference vessel diameter	4.57 (0.49)	4.96 (0.3)	< 0.0001
Per cent diameter stenosis	6.58 (16.57)	10.18 (8.07)	0.014
Late lumen loss		-0.115 (0.26)	

RESULTS

Angiography and QVA

All stents were successfully implanted and conformed well to the vessel anatomy at implantation. There were no cases of thrombus formation, edge dissections, or slow-flow. All stents showed optimal trackability, pushability, and angiographic visibility. After 28 days in angiography, all treated vessels were patent, with no signs of excessive neointimal hyperplasia or thrombus formation. The QVA analysis is presented in Table 1. During 28-day follow-up, the vessel reference diameter increased by 10% ($p < 0.05$), which also translated into negative late lumen loss.

Pathological analysis

In the histopathological analysis, all stents were patent, fully covered with neointima, with no signs of luminal thrombi. Morphometric evaluation shows mean area stenosis of $17.4 \pm 9.0\%$, mean intimal thickness of 0.2 ± 0.15 mm, and luminal area of 12.19 ± 1.0 mm² (Table 2). The qualitative assessment of healing and biocompatibility shows low injury

Table 2. Histomorphometry

EEL area [mm ²]	17.87 ± 2.79
IEL area [mm ²]	14.99 ± 2.81
Medial area [mm ²]	2.89 ± 0.25
Intimal area [mm ²]	2.8 ± 2.28
Luminal area [mm ²]	12.19 ± 1.0
Area stenosis [%]	17.4 ± 9.0
Mean intimal thickness [mm]	0.2 ± 0.15

EEL — external elastic lamina; IEL — internal elastic lamina

and inflammation score (Table 3). In descriptive histology, macrophages and rare multinucleated giant cells (MGCs) were observed in the majority of samples. In one stent, as well as macrophages and MGCs, granulomas containing abundant neutrophils were observed. One stent had medial mineralisation, which is a common reaction of the media to injury. Many stents had medial inflammation, but the mean severity grade was low. Two stents had evidence of neointimal

Table 3. Histopathology scores

Injury score	0.34 ± 0.36
Inflammation score	0.58 ± 0.23
Fibrin score	0.18 ± 0.18
Endothelialisation score	3.0 ± 0.0
Neointimal smooth muscle score	1.97 ± 0.37

immaturity with moderate mean severity grade, and one stent had evidence of medial immaturity with mild severity grade. Three stents had moderate adventitial inflammation and fibrosis, suggesting chronic injury or irritation.

DISCUSSION

CAS is a rapidly developing technique that has shown comparable late outcomes in comparison with CAE [7]. Periprocedural stroke is a significant limitation of CAS. In the CREST trial CAS was complicated by stroke in 4% of cases [3]. The improvement of stent technology and operator experience may significantly improve periprocedural outcomes.

The improvement of carotid stents may be achieved by redesigning the stent architecture. The closed cell stent design provides better scaffolding due to a higher percentage of arterial wall coverage. Bosiers et al. [5] suggested that a smaller free cell area provided by closed cell design may prevent periprocedural ischaemic events. This is especially important in unstable lesions with soft and highly thrombogenic plaques. Nonetheless, open cell stent design ensures better deliverability and conformability to carotid anatomy.

The assayed stent has a hybrid cell design, which provides excellent deliverability and conformability with decent coverage of the lesion by the stent area. This technology incorporates the advantages of open and closed cell stent technology. Moreover, nitinol is a proven material used for medical implants, with desirable physical properties like super elasticity [8]. All these properties are necessary in the tortuous and narrow anatomy of carotid bifurcation. The electro-polished surface of the stent additionally enhances its radial strength and minimises cracks and grooves in the layer [9].

The results from QVA are very reassuring with regard to acute and late outcome. At 28 days, the vessels were patent, without signs of thrombi, aneurysm, or excessive neointimal proliferation. Interestingly, there was a 10% temporal increase in vessel reference diameter and negative late lumen loss. This is a common and desired feature of self-expanding stents, which allows compliance to vessel anatomy over time and was confirmed with the studied device. Histopathology shows good biocompatibility and vascular healing profile as evidenced by very low value of injury, inflammation, and fibrin scores. A high rate of those parameters is usually associated with restenosis caused by excessive neointimal growth [10]. Moreover, histopathology has shown complete endothelialisation,

which is considered as a deterrent of stent thrombosis. Complete endothelialisation of bare metal stents implanted in humans is typically confirmed by necropsy reports within the period of three to four months [11]. In descriptive histology, the vast majority of struts have infiltration of macrophages and MGCs. Those cells represent a chronic reaction of the artery to a foreign body, which is the stent strut [12]. The neutrophil infiltration found in one stent is related to acute inflammation and is rarely observed beyond one month [13]. These histological findings, presenting favourable vascular response to tested stents and good healing profile of treated arteries, may translate into low clinical risk of serious adverse events including thrombosis and restenosis.

To our best knowledge, other studies of hybrid carotid stents have not been published yet. In comparison to commercially available self-expandable nitinol stents dedicated for carotid arteries (Precise[®] Corids, USA), MER[®] has had similar outcomes in preclinical studies [13, 14].

Limitations of the study

This study has several limitations. The differences in anatomy and physiology of healthy carotid arteries in pigs and humans with atherosclerosis may cause some discrepancies in terms of efficacy found in clinical and preclinical studies. Nonetheless, the pig is suitable and widely accepted model for preclinical stent testing [15]. Another limitation is the short observation period of only four weeks. Nevertheless, this period covers the most important time for bare metal stents in terms of healing, endothelialisation, and, most frequent in this time, thrombotic events. Another issue is the lack of a control group; however, the aim of the study was the assessment of safety and biocompatibility — we did not assume to control efficacy. It must be pointed out that the transfer of results to humans is restricted but must be undertaken before first-in-man studies [14].

CONCLUSIONS

The MER[®] — a new self-expanding nitinol stent dedicated for carotid arteries — is safe and biocompatible. The hybrid design, fast endothelialisation, and minimal inflammatory reaction may contribute to favourable clinical results.

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Conflict of interest: Krzysztof Milewski — consultant for Balton Sp. z o.o.

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Bezpieczeństwo i biokompatybilność nowego samorozprężalnego stentu nitinolowego zbudowanego w technologii komórek hybrydowych przeznaczonego do tętnic szyjnych na modelu świni domowej

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Streszczenie

Wstęp: Stenty przeznaczone do rewaskularyzacji tętnic szyjnych zbudowane w technologii otwartokomórkowej charakteryzują się stosunkowo łatwym dopasowaniem do anatomii leczonego segmentu, podczas gdy technologia zamkniętokomórkowa zapewnia lepszą stabilizację blaszki miażdżycowej.

Cel: Celem badania była ocena możliwości stosowania i odpowiedzi biologicznej ściany naczynia na nowy stent samorozprężalny przeznaczony do tętnic szyjnych (MER[®], Balton, Polska), wykonany w technologii komórek hybrydowych, łączących zalety stentów otwarto- i zamkniętokomórkowych, na modelu świni domowej.

Metody: Badane stenty (n = 10) zostały implantowane do tętnic szyjnych świń przy użyciu zwalidowanego i powszechnie stosowanego modelu uszkodzenia ściany naczynia (przerozmiarowanie średnicy stentu w stosunku do średnicy referencyjnej naczynia o ok. 10%). Kontrolna angiografia została wykonana przed, bezpośrednio po implantacji oraz w 28. dniu eksperymentu. W ostatnim dniu badania segmenty tętnic szyjnych wraz ze stentami wyizolowano i przekazano do analizy histopatologicznej.

Wyniki: Wszystkie stenty zostały łatwo wprowadzone oraz implantowane do wybranych segmentów tętnic szyjnych. Kontrolna angiografia uwidoczniała dobrą apozycję stentów, brak ich złamań i brak nadmiernego przerostu neointimy. Utrata światła naczynia w analizie angiografii ilościowej po 28 dniach wyniosła $-0,11 \pm 0,3$ mm, a odsetek zwężenia średnicy światła tętnicy wyniósł $10,18 \pm 8,1\%$. Referencyjna średnica światła naczynia zwiększyła się o 10% ($4,57 \pm 0,5$ vs. $4,96 \pm 0,3$ mm, $p < 0,01$). Średnia powierzchnia zwężenia wynosiła 17,4%, a średnia grubość intymy — 0,2 mm. W badaniu histopatologicznym parametry, takie jak uszkodzenie naczynia, obecność złogów fibryny i odczyn zapalny były niskie. Endotelializacja we wszystkich stentach była pełna, a stopień dojrzałości neointymy oceniono jako średni. Tylko w 1 stencie zaobserwowano naciek granulocytarny, a w innym mineralizację wokół przęseł.

Wnioski: Nowy polski samorozprężalny stent nitinolowy wykonany w technologii komórek hybrydowych charakteryzuje się optymalną biokompatybilnością i szybkim gojeniem ściany tętnicy. Na tej podstawie może zostać wdrożony do pierwszego użycia w praktyce klinicznej.

Słowa kluczowe: stentowanie tętnic szyjnych, nowe technologie, badania przedkliniczne

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