Noble Metal Nanoparticles for Plasmonic Photothermal Therapy of Atherosclerosis

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ABSTRACT

Atheroregression becomes an attractive target for cardiovascular treatment. Some clinical trials have demonstrated that intensive therapy with rosuvastatin or recombinant ApoA-I Milano can partially reduce the total atheroma volume (TAV) up to 6.38 mm^3 or 14.1 mm^3 respectively. Our previous bench studies of selected nanotechnologies documented TAV reduction up to unprecedented 79.4 mm^3. The completed observational three arms (n=180) first-in-man trial (the NANOM FIM trial) assessed (NCT01270139) the safety and feasibility of two delivery techniques for nanoparticles (NP), and plasmonic photothermal therapy (PPTT). Patients were assigned to receive either (1) nano-intervention with delivery of silica-gold NP in bioengineered on-artery patch (n=60), or (2) nano-intervention with delivery of silica-gold iron-bearing NP with targeted micro-bubbles or stem cells using magnetic navigation system (n=60) versus (3) stent implantation (n=60). The primary outcome was TAV at 12 months. The mean TAV reduction at 12 months in Nano group was 60.3 mm^3 (SD 39.5; min 41.9 mm^3, max 94.2 mm^3; $p<0.05$) up to mean 37.8% (95% CI: 31.1%, 51.7%; $p<0.05$) plaque burden. The analysis of the event free survival of the ongoing clinical follow-up shows the significantly lower risk of cardiovascular death in Nano group when compared with others (91.7% vs 81.7% and 80% respectively; $p<0.05$) with no cases of the target lesion-related complications. So, PPTT using silica-gold NP associated with significant regression of coronary atherosclerosis.

Keywords: Glagov phenomenon, atheroregression, plasmonics, nanotechnology.

1 INTRODUCTION

The reversal of atherosclerosis below becomes a new attractive target for cardiovascular therapy and coronary device development[1, 2]. Some clinical trials have demonstrated that lowering low density lipoprotein (LDL) levels through intensive statin therapy while accompanied by raised high density lipoprotein (HDL), can slow progression, or even partially reduce the total atheroma volume (up to 6.38 mm^3) in coronary arteries[1, 2]. Of note, plaque regression was associated only with a 30% relative reduction in events. By the way of comparison, recombinant ApoA-I Milano demonstrated a 14.1 mm^3 reduction in total atheroma volume[3]. Plasmonic photothermal therapy (PPTT) using near-infrared (NIR) laser irradiation[4-6] is the novel invasive approach in cardiology. The noble-metal nanoparticles are the only type of optically active composite spherical particles on the nanoscale[7-9] for needs of PPTT. Our previous bench studies PLASMONICS[10] documented acceptable level of safety and significant efficacy of PPTT with unprecedented plaque burden (PB) reduction up to 79.4 mm^3.

The first-in-man trial (the NANOM FIM trial) assessed the safety and feasibility of two delivery techniques for NP, and PPTT of atherosclerotic lesions in patients with coronary artery disease (CAD) and SYNTAX (SYNergy between percutaneous coronary intervention with TAXus and cardiac surgery) score ≤22.

2 METHODS

This trial was a multi-center (two sites), observational, open-label, three arms study in 180 patients with CAD and angiographic SYNTAX score ≤22. The protocol was approved by the Ethics Committee and the Research Steering Committee of the Ural State Medical University (Yekaterinburg, Russia). The study is registered in clinicaltrials.gov with identifier NCT01270139. All participants signed written informed consent before enrollment. The project started in April, 2007 and completed in June, 2012. After the technology development in September 2004, a series of animal experiments[10] has described as PLASMONICS study were performed in 2004-2007. On the basis of excellent experimental results, the ethics committee of the Ural Institute of Cardiology (Yekaterinburg, Russia) under the supervision of the Medical University permitted the start of human interventions.

Diseased patients, men and women, aged 45-65 years were enrolled if they were judged to have single- or multi-vessel CAD with flow-limiting lesions and without indications for coronary artery bypass surgery (CABG), stable angina with indications for percutaneous coronary interventions (PCI), NYHA (New York Heart Association) I-III functional class of heart failure (HF), treated hypertension (in supine position: systole >140 mm Hg, diastole >90 mm Hg). All patients with history of PCI were allocated if they did not have anamnesis of myocardial infarction (MI). Exclusion criteria included non-compliance, angiographic SYNTAX score ≥23, history of MI, unstable angina, or CABG, atrial fibrillation or other arrhythmias, stroke; indications for CABG, contraindications for PCI or CABG; NYHA IV functional class of HF, diabetes mellitus (in case of fasting glucose >7.0 mM/L or random glucose >11.0 mM/L), untreated hypertension, asthma, known hypersensitivity or contraindications to anti-platelet drugs,

contrast sensitivity, and participation to any drug- or intervention-investigation during the previous 60 days.

Target lesions were selected if they had less than 3.0 mm in diameter and 10 mm in length by visual estimation, a stenosis of between 50% and 99% of luminal diameter with a TIMI (Thrombolysis in Myocardial Infarction) flow grade of 1 or more with a total atheroma volume (TAV) of the target lesion at least 100 mm^3 (minimal vessel volume – at least 200 mm^3) as assessed by online quantitative coronary angiography (QCA) or intravascular ultrasound (IVUS). Patients were ineligible if they had any of the following: left main coronary artery stenosis, an ostial lesion, lesion located within 2 mm of a bifurcation; lesion with moderate-to-heavy calcification by visual assessment; angiographically visible thrombus within the target lesion.

Patients who met the inclusion criteria were assigned (the intention-to-treat population, ITTP) in a 1:1:1 ratio to receive either (1) nano-intervention with delivery of silica-gold nanoparticles (NP) in mini-surgery implanted bioengineered on-artery patch (n=60), or (2) nano-intervention with delivery of silica-gold iron-bearing NP with targeted micro-bubbles or stem cells using magnetic navigation system (n=60).

In control group, XIENCE V stent (Abbott Vascular, Santa Clara, CA) was implanted to 60 patients. Patients with a single de novo native coronary stenosis were stented by a single stent of 3.0 x 18 mm. The implantation had to be performed according to common interventional practices including the administration of intracoronary nitroglycerine 0.2 mg of glycerol trinitrate or isosorbide dinitrate and intra-arterial heparin (50-100 U/kg body weight). Predilation with a conventional balloon catheter (with a pressure not exceeding the rated-burst pressure 16 atm) was recommended before drug-eluting stent (DES) deployment according to the manufacturer's recommendation. The protocol recommended the study stent should cover 2 mm of non-diseased tissue on either side of the target lesion. Postdilatation was allowed with a balloon that was shorter than was the study device.

Some patients were excluded from the per-treatment-evaluable population (PTEP) if they had a history of PCI, or received a stent in addition to the study device (if interpreted as target vessel revascularization - TVR), or were treated with CABG (if interpreted as target lesion revascularization - TLR). Patients were also excluded from PTEP in case of non-compliance or documented diabetes, NYHA IV functional class of HF, or SYNTAX score ≥23. The ITTP population was our primary population.

The primary outcome was TAV (plaque-media volume, mm^3) at 12 months. The secondary outcomes were per cent atheroma volume (PAV, plaque burden, %), composition of plaque (per cent of fibro-fatty component, per cent of fibrous component, per cent of necrotic core, per cent of calcium), minimal lumen diameter (MLD, mm), event free survival, TLR, restenosis rate (stenosis >50%), late definite thrombosis rate, and coronary vasomotion at 12 months. Imaging and clinical end-points were assessed pre-, post-procedure and at 12-month follow-up.

We defined a clinical device success as successful delivery of nanoparticles (NP) or implantation of stent at the target lesion. The procedure of NP delivery was IVUS- and QCA-guided. Clinical procedure success was defined as above without the occurrence of major adverse clinical events related to ischemia up to 7 days after the index procedure. All major adverse cardiac events were adjudicated by an independent clinical events committee, and a data safety monitoring board monitored patient safety. Ten per cent of QCA and IVUS data were analyzed by independent CoreLab (the Heart Clinic, Yekaterinburg, Russia). The SYNTAX score was calculated using the calculator which is available online (www.syntaxscore.com). All variables pertinent to calculation were computed by three interventional cardiologists who were blinded to procedural data and clinical outcome.

3 RESULTS

The procedure was a success in all 180 patients, and device success (calculated on a per-device basis) was 100%. The minimum duration of follow-up was 369±10 days. All the patients were thoroughly tested before intervention in order to reduce a risk of the procedure-related complications and optimize results. Up to 12 months we recorded no instances of life-threaten complications or thrombosis at the site of intervention.

The reduction of the PAV (PB) post-procedure/ at 12-month follow-up was 12.6%/44.8%, 13.1%/43.2%, 20.2%/22.7% ($p<0.05$ for comparisons between two nano-groups and control) in Nano, Ferro and Control groups of the ITTP respectively. The mean TAV reduction in Nano group was 60.3 mm^3 (SD 39.5; min 41.9 mm^3, max 94.2 mm^3).

The increase in MLD from baseline to post-procedure/ at 12-month follow-up was 5.9/ 63.6%, 21.2/ 63.8%, 56.2/ 62.4% in groups respectively. Inspite of the 'bumpy' and dilative changes in the lumen dimensions, we have documented only one case of the definite thrombosis at the site of intervention in Ferro group with three events of the TLR in both Nano and Ferro groups. IVUS analysis also has shown the late lumen enlargement with signs of proper endothelial recovery and progressive restoration of the injured artery wall.

The serial assessments of VH-IVUS showed a significant decrease at 12 months in the dense calcium area, fibrous and fibro-fatty tissue in nano groups, whereas we measured an increase of fibrous and fibro-fatty components in stenting control group. Our observations document significant increase in necrotic core post-procedure with further reduction in size at 12-month follow-up.

There was statistical heterogeneity of hazard ratio between prespecified subgroups for the primary end point, with greater regression of PAV in Nano group as compared with Ferro or control groups in patients with smaller baseline size of necrotic core ($p=0.03$) and larger baseline fibro-fatty component ($p=0.04$).

For 120 patients in both studied groups at 12 months we recorded binary stenosis in four patients (3.3%) - two

cases per group. The Kaplan-Meier analysis of the event free survival of the ongoing clinical follow-up shows the significantly lower risk of cardiovascular death in Nano group if compare with others (91.7% vs 81.7% and 80% respectively; $p<0.05$). But five cases of death as well as three cases of myocardial infarction in Nano group were not associated with target lesion. One patient was stented with XIENCE V stent at the target lesion due to restenosis at 11 months after the intervention.

All patients were undergone vasomotion tests. Overall, the increase in the mean lumen diameter after Ach administration was 2.89% ($p=0.053$), 3.84% ($p=0.058$), and 1.04% ($p=0.12$) in groups respectively. 178 patients (98.9%) exhibited a slight vasodilatory response to Ach, whereas 2 patients (1.1%) had an abnormal response to Ach with vasoconstriction. The analysis of cumulative frequency for the changes in mean lumen diameter shows significantly ($p<0.0001$) higher vasodilatory sensitivity of arteries to Ach in nano groups if compare with stenting control.

We did not document any changes in the level of cholesterol at 12 months with concomitant treatment by rosuvastatin 40 mg orally daily. The level of LDL (low-density lipoprotein) cholesterol has been changed from 1.64 (95% CI: 1.61-1.78) to 1.78 (95% CI: 1.66-1.80) mmol per liter at 12 month after the nano-intervention or stenting ($p>0.05$). The HDL (high-density lipoprotein) cholesterol decreased from 1.32 (95% CI: 1.30-1.34) to 1.29 (95% CI: 1.27-1.33) mmol per liter ($p>0.05$ for all comparisons) in groups.

4 DISCUSSION

Current PCI using DES generally just manipulates the form of the plaque and has some clinical and technical limitations as well as relatively high complication rate[1]. As in the porcine model with bioresorbable scaffolds (BRS) implantation, late lumen enlargement, and plaque-media reduction (12.7%) with wall thinning were observed in humans using IVUS[2, 10, 12, 13]. By comparison, in the most recent study of plaque regression in patients receiving rosuvastatin, the relative reduction in plaque-media volume was 8.5% over a period of 2 years[1, 2]. Numerous devices utilizing heat and high-energy light such as laser technology (excimer ultra-violet laser)[14], electrosurgical approach, radio frequency sparking have been also described as the applications to treat atheroma. Plasmonics offers a novel solution for atherodestruction heralding a new era of the manageable atheroregression in cardiology with possibility to reverse atherosclerosis.

Our previous bench studies[10] in Yucatan mini-swines have established feasibility and significant benefit of PPTT for atheroregression with mean reduction of PB up to 79.4 mm^3, acceptable level of safety, and no evidences of cytotoxicity. Among five available techniques for delivery of NP we have selected three approaches with maximal safety that use (1) transcatheter intravascular infusion or injection to the lesion of the pre-incubated with NP autologous stem cells, (2) mini-invasive cardiac surgery transplantation of bioengineered on-artery patch (to the projection of culprit lesion) grown with stem cells bearing NP, and (3) transcatheter intravascular infusion or injection of the iron-bearing NP with stem cells or targeted protein-coated microbubbles, and delivery using magnetic fields. Last two techniques demonstrated the relatively high accumulation of NP in the target lesion and low risk of acute plaque rupture or acute thrombosis at the site of intervention that was warranted and deemed appropriate for further tests in humans.

This study compared two different approaches for delivery of NP with the main goal to destruct the target atheroma with acceptable for the real clinical practice level of safety and efficacy. The regression of PB was achieved in both experimental groups with high level of safety, unprecedented reduction of TAV up to 60 mm^3 (more than 92% of patients in Nano group), and late lumen enlargement without signs of positive or negative artery remodeling.

Recently cardiology is searching for an optimal target in treatment of atherosclerosis – from the routine restoration of the lumen dimensions and delaying of atherogenesis to atheroregression and reparative vascular therapy. Currently, the maximum success of conventional therapy with drugs was documented on the level of 6 mm^3 with rosuvastatin[1], and a 14 mm^3 - in studies of ApoA-I Milano[3]. Definitely, this is about incomparable populations of patients, but these two examples demonstrate very well a certain threshold for current therapeutic approaches. PPTT gives a chance to conquer the atherosclerosis, dramatically decreasing TAV, reducing cardiovascular mortality and improving quality of life. In fact, our study registered no target lesion major adverse cardiac events (MACE). The precise nature of the relationship between prevention of MACE and PB reduction remains a subject of ongoing research. Theoretically, regression involves reductions of the lipid, inflammatory, and necrotic components of plaque, each of which has been implicated in plaque rupture. And indeed our study documented reduction of the necrotic core, fibrous and fibro-fatty components as well as dense calcium by results of VH-IVUS analysis at 12-month follow-up.

The explanation of the working mechanism for PPTT is a major challenge in the current studies. The influence of the following tissue-destructive factors of PPTT, which was called 'cooking' with NIR laser[6, 7, 9], is expected: high-heat plasmonic detonation of nanoshells (Au atoms and clusters got a high velocity) with thermo-mechanical damage of targeted tissues, vapor bubbling of cellular cytoplasm and extracellular matrix with subsequent degradation and melting of tissues, and destructive effects of acoustic and shock waves with supersonic expansion. There are two main physical mechanisms that could lead to the laser-induced explosion of nanoparticles - thermal explosion mode through electron-photon excitation-relaxation, and Coulomb explosion mode through multiphoton ionization. Thermal explosion mode ('nanobombs') of PPTT implies a thermal explosion of nanoparticles which occures when heat is generated within the strongly-absorbing target more rapidly than the heat can diffuse away. VH-IVUS analysis in our study demonstrated significant enhancement of the necrotic

core immediately after the NP detonation that correlates with above-described plasma-thermolytic mechanism of the PPTT. The clinical value of PPTT in case of the vulnerable plaque with large necrotic core and thin cap is another target for the running clinical trials.

The potential for restoration of vasomotion at 12 months with predominantly vasodilative response to Ach hypothetically underlines the recovery of the vessel wall architecture after PPTT with proper re-endothelialization and physiological function of smooth muscle cells. The mechanism of the tissue repair after the intervention requires further bench investigations. Injured or 'burned' areas probably were restored by 'inflammatory' and stem or progenitor cells from the resident tissues, niches in adventitia and circulation. A role of transplanted allogenious stem cells as both carriers of NP and bioactive substances for the local regulation of tissue repair still requires clarification in the pre-clinical investigations. The reduction of the dense calcium by VH-IVUS data is also an argumentative evidence of the stem cell benefits for the physiological remodeling of the artery after aggressive PPTT.

One of the key limitations of this approach is the optimal technique of the NP delivery into the target tissue of the culprit atheroma. The delivery of NP with bioengineered patch has had more pronounced effect for atheroregression with more significant level of safety. Potentially, both studied approaches of delivery are of great importance for the further clinical development, but still being relatively aggressive and traumatic for patients. The potential progress of the proposed technology includes development of two different approaches for delivery with utilization of stem cells as the main carriers for NP. The first approach is more applicable for patients with indications for PCI and implicates micro-infusion trans-catheter (Cricket or Bullfrog micro-infusion catheters of Mercator Medical Systems, San Leandro, CA) intramural injection of stem cells into the artery or perivascular tissues under the control of the advanced imaging. The second strategy concerns patients with indications for CABG, and includes growing of the bioengineered patch as a carrier for stem cells with the subsequent MICS CABG transplantation.

An absence of the solution to acutely or urgently maintain the lumen remains another technical and clinical limitation for above-mentioned nano-approach. Both the transient implantation of BRS and high-energy NIR laser angioplasty represent potentially very elegant tools for real clinical practice in case when the urgent restoration of blood flow is required. The combination of NIR angioplasty (maintain of the lumen) with NIR spectroscopy (intravascular imaging) and NIR laser detonation of NP inside the lesion (management of the entire plaque) is able to secure the high clinical theranostic value of NIR technologies.

5 CONCLUSION

Plasmonic resonance therapy using silica-gold NP associated with significant regression of coronary atherosclerosis. Both approaches for delivery of NP have acceptable for clinical practice level of safety as well as similar degree of regression of TAV in favor of the minimally invasive cardiac surgery implantation of the bioengineered patch onto the artery.

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