CLINICAL RESEARCH

Cutting balloon combined with paclitaxel-eluting balloon for treatment of in-stent restenosis

Cutting balloon combiné à un ballon paclitaxel pour le traitement de la resténose intra-stent

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KEYWORDS
In-stent restenosis; Angioplasty; Animal model; Apoptosis

Summary
Background. — The optimal therapy for in-stent restenosis (ISR) is controversial. We evaluated three different strategies for the treatment of in-stent restenosis: cutting balloon angioplasty (CBA), paclitaxel-eluting balloon angioplasty (PEBA) and cutting balloon followed by paclitaxel-eluting balloon angioplasty (CB + PEBA).
Methods. — Forty-five coronary arteries in 45 mini-pigs underwent oversized bare-metal stent (stent-to-artery ratio, 1.2:1) implantation to induce in-stent restenosis. After 28 days, vessels with in-stent restenosis (≥50% diameter stenosis) were randomly divided into three groups: CBA, PEBA and CB + PEBA. In vivo angiography was performed before intervention, immediately after intervention and at 28-day follow-up. Stented arteries were harvested for pathological

Abbreviations: AS%, percentage of lumen area stenosis; BMS, bare-metal stent; CBA, cutting balloon angioplasty; CB + PEBA, cutting balloon followed by paclitaxel-eluting balloon angioplasty; EELA, elastic lamina area; IELA, internal elastic lamina area; ISR, in-stent restenosis; LA, lumen area; LD, lumen diameter; NA, neointimal area; PCNA, proliferating cell nuclear antigen; PEBA, paclitaxel-eluting balloon angioplasty; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labelling; VSMCs, vascular smooth muscle cells.

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Introduction

Treatment of in-stent restenosis (ISR) remains challenging. Several studies have shown variable results using balloon angioplasty alone [1], repeat stenting [2—4], cutting balloon angioplasty (CBA) [5,6], intracoronary irradiation (brachytherapy) [7,8] or excimer laser angioplasty [9]. Other studies have compared these different techniques and it is still unclear which one, if any, will provide the most favourable outcomes [10—15].

Recently, paclitaxel-eluting balloon angioplasty (PEBA) has been developed as a novel approach, which combines the features of conventional balloon angioplasty with paclitaxel eluting for the treatment of ISR. Preclinical trials have demonstrated that the efficacy of PEBA in the treatment of ISR is superior to that of conventional balloon angioplasty and not inferior to that of a paclitaxel-eluting stent [16,17]. However, given the structure of the paclitaxel-eluting balloon, it must have some of the shortcomings of conventional balloon angioplasty, such as balloon slippage and edge dissections post procedure. All these shortcomings have been associated with cumbersome procedures, suboptimal results and adverse clinical and angiographical outcomes [18]. The use of CBA could potentially reduce the occurrence of these complications. However, the outcome of cutting balloon predilatation followed by PEBA for the treatment of ISR compared with PEBA or CBA alone is unknown.

analyses. The proliferation and apoptosis of vascular smooth muscle cells were evaluated by immunohistochemical staining and the terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) assay, respectively.

Results. — Acute lumen gain was not different between the three groups. Late lumen loss and neointimal area at follow-up were lower for CB + PEB in comparison with CBA but similar for CB + PEB compared with PEB in group. There were no significant differences in proliferating cell nuclear antigen-positive vascular smooth muscle cells and TUNEL-positive vascular smooth muscle cells between the CB + PEB and PEB groups.

Conclusions. — PEBA with or without cutting balloon was superior to CBA alone for in-stent restenosis. The underlying mechanism was probably related to inhibition of smooth muscle cell proliferation and increased apoptosis. In this porcine coronary artery restenosis model, PEBA with or without cutting balloon was superior.
The aims of this study are to compare 28-day imaging and pathology outcomes between PEBA, CBA and cutting balloon followed by paclitaxel-eluting balloon angioplasty (CB + PEBA) for ISR in pig models and to elucidate the possible mechanism.

Methods
All animal care and procedures conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and were approved by the Institutional Animal Care and Use Committee of the Second Affiliated Hospital of Harbin Medical University (2009-X023).

Establishment of the in-stent restenosis model
Forty-five mini-pigs (20–25 kg) were pretreated with aspirin (300 mg) and clopidogrel (75 mg) once a day starting 3 days prior to the procedure. Animals were intubated after sedation with ketamine (20 mg/kg, intramuscularly) and diazepam (0.4 mg/kg, intramuscularly), followed by 3% sodium pentobarbital through the marginal ear vein (25 mg/kg, intravenously). A 6-F guiding catheter was used. Continuous haemodynamic and surface electrocardiographic monitoring was maintained throughout the procedure. After intravenous heparin (150 U/kg) and intracoronary injection of nitroglycerin (100 μg), baseline angiography of the target vessel was performed. The methods of stent implantation have been published previously [19]. One bare-metal stent (BMS) (3.0–3.5 × 18 mm; Lepu Medical Company, Beijing, China) was placed at 12–14 atm for 30 s in the left anterior descending artery of each pig. The stent-to-artery ratio was maintained at 1.2:1. After the equipment was removed, all mini-pigs were sent back to the animal house, where they were fed a normal diet, and received aspirin (300 mg, orally) and clopidogrel (75 mg, orally) daily.

Interventional procedure for in-stent restenosis
At 28 days after BMS implantation, a repeat angiography was performed. The segments with ISR (> 50% diameter stenosis) by quantitative coronary angiography were randomly assigned to one of the three treatment groups: CBA, PEBA or CB + PEBA.

Cutting balloon angioplasty
The length of the cutting balloon (Boston Scientific, Natick, MA, USA) was 10–15 mm and the diameter was chosen according to the size of the stent (cutting balloon-to-stent ratio, 1:1:1). The cutting balloon was positioned at the lesion site and inflated to the recommended maximal pressure of 10 atm once.

Paclitaxel-eluting balloon angioplasty
Predilation of the target lesion was usually required before placement of the study device. The diameter of the conventional non-study balloon catheter was 0.5 mm smaller than that of the paclitaxel-eluting balloon. The paclitaxel-eluting balloon (3.0–3.5 × 20 mm; SeQuent Please, BRAUN, Germany) was inflated in the same fashion as a conventional balloon catheter for 60 s and the pressure was 10 atm.

Cutting balloon followed by paclitaxel-eluting balloon angioplasty
Before the paclitaxel-eluting balloon was inflated, predilation of the target lesion was carried out with a cutting balloon catheter, which was similar in diameter to a paclitaxel-eluting balloon.

Quantitative coronary analyses
Angiograms were performed during the initial procedure, on day 28 after BMS implantation and 28 days after three different strategies for the treatment of ISR. A computerized coronary angiography analysis system (GE Company, Germany) was used for quantitative coronary analyses by two experienced cardiologists blinded to the treatment protocol. Discrepancies were resolved by mutual consensus. The immediate lumen diameter (LD) gain (minimum LD immediately after the interventional procedure minus minimum LD before the interventional procedure) and late lumen loss (minimum LD immediately after the interventional procedure minus minimum LD at follow-up) were calculated.

Pathological evaluation
For the morphometric analysis, stented arteries were harvested and fixed in 10% buffered formalin and embedded in glycol methacrylate. Stented segments were cut into three parts (proximal, mid and distal). Thin sections from each artery block were stained with haematoxylin and eosin and Verhoeff van Gieson for measurement of the external elastic lamina area (EELA), lumen area (LA) and internal elastic lamina area (IELA). The neointimal area (NA) was calculated using the equation $\text{NA} = \text{IELA} - \text{LA}$. The percentage of lumen area stenosis (AS%) was calculated using the equation $\text{AS}\% = (\text{NA}/\text{IELA}) \times 100$. Injury score at each strut site was assessed as described by Schwartz et al. [20], where: 0 = no injury; 1 = break in the internal elastic membrane; 2 = perforation of the media; and 3 = perforation of the external elastic membrane to the adventitia. The mean injury score for each segment was calculated by dividing the sum of injury scores by the total number of struts at the examined section.

Stent wires were carefully removed and the tissue was embedded in paraffin. Vessel-wall expression of proliferating cell nuclear antigen (PCNA) was evaluated by immunohistochemical analyses (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Apoptosis of vascular smooth muscle cells (VSMCs) was evaluated by the terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) assay. There were three cross-sections of each arterial specimen to analyse. The samples were incubated, developed, and counterstained with haematoxylin. The total number of VSMCs in each randomly chosen neointimal cross-section was measured with computer assistance at $\times 400$ magnification (Pro Plus 5.0 software). The percentages of proliferating or apoptotic VSMCs were obtained by dividing the number of
PCNA- or TUNEL-positive VSMCs by the total number of VSMCs in each cross-section; the results were then averaged.

Statistical analyses

Data are expressed as means ± standard deviations except where noted. The mean angiographical, histological and morphological data for each stent were compared by one-way analysis of variance with post hoc analysis for multiple comparisons. A value of $P < 0.05$ was considered significant.

Results

Forty-five mini-pigs underwent successful implantation of 45 BMSs in the left anterior descending artery. All mini-pigs survived until the end of experiments. Thirty-one out of 45 lesions (68.9%) had greater or equal to 50% diameter stenosis and the mean angiographical stenosis was $62.2 \pm 10.3\%$.

Table 1  Quantitative coronary angiography results.

<table>
<thead>
<tr>
<th>Variables</th>
<th>CBA $(n=11)$</th>
<th>PEBA $(n=10)$</th>
<th>CB + PEBA $(n=10)$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reference diameter (mm)</td>
<td>2.67 ± 0.24</td>
<td>2.52 ± 0.18</td>
<td>2.59 ± 0.25</td>
<td>0.8951</td>
</tr>
<tr>
<td>Stent diameter (mm)</td>
<td>3.21 ± 0.34</td>
<td>3.12 ± 0.22</td>
<td>3.18 ± 0.28</td>
<td>0.912</td>
</tr>
<tr>
<td>Balloon-to-artery ratio</td>
<td>1.24 ± 0.06</td>
<td>1.25 ± 0.02</td>
<td>1.22 ± 0.08</td>
<td>0.6312</td>
</tr>
<tr>
<td>MLD (mm)</td>
<td>1.01 ± 0.28</td>
<td>1.06 ± 0.23</td>
<td>1.05 ± 0.41</td>
<td>0.5413</td>
</tr>
<tr>
<td>Diameter stenosis (%)</td>
<td>60.2 ± 10.1</td>
<td>61.2 ± 7.5</td>
<td>63.7 ± 12.1</td>
<td>0.6338</td>
</tr>
<tr>
<td>Post intervention</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reference diameter (mm)</td>
<td>2.65 ± 0.19</td>
<td>2.61 ± 0.22</td>
<td>2.65 ± 0.28</td>
<td>0.796</td>
</tr>
<tr>
<td>MLD (mm)</td>
<td>2.15 ± 0.14</td>
<td>2.17 ± 0.13</td>
<td>2.19 ± 0.35</td>
<td>0.765</td>
</tr>
<tr>
<td>Acute lumen gain (mm)</td>
<td>1.12 ± 0.36</td>
<td>1.12 ± 0.16</td>
<td>1.15 ± 0.41</td>
<td>0.785</td>
</tr>
<tr>
<td>Follow-up at 28 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reference diameter (mm)</td>
<td>2.63 ± 0.15</td>
<td>2.61 ± 0.17</td>
<td>2.65 ± 0.20</td>
<td>0.8125</td>
</tr>
<tr>
<td>MLD (mm)</td>
<td>1.25 ± 0.57</td>
<td>2.04 ± 0.14$^*$</td>
<td>2.05 ± 0.38$^*$</td>
<td>0.003</td>
</tr>
<tr>
<td>Late lumen loss (mm)</td>
<td>0.89 ± 0.63</td>
<td>0.13 ± 0.04$^*$</td>
<td>0.11 ± 0.05$^*$</td>
<td>0.0406</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard deviation. CBA: cutting balloon angioplasty; CB + PEBA: cutting balloon followed by paclitaxel-eluting balloon angioplasty; MLD: minimal luminal diameter; PEBA: paclitaxel-eluting balloon angioplasty. Late lumen loss: post intervention MLD — follow-up MLD. A $P$ value < 0.05 indicates a significant difference.

* $P < 0.05$ compared with CBA.

All statistics were calculated with SPSS version 18.0 software.

Figure 1. Morphometric analyses at follow-up (28 days) of three interventional groups for in-stent restenosis. Haematoxylin and eosin staining ($\times$ 20) of cross-sections of coronary arteries treated with (A) cutting balloon angioplasty, (B) paclitaxel-eluting balloon angioplasty and (C) cutting balloon followed by paclitaxel-eluting balloon angioplasty.
At the end of the experiments, there were 11 lesions in the CBA group, 10 lesions in the PEBA group and 10 lesions in CB + PEBA group.

**Angiographical findings**

Quantitative coronary angiography results are summarized in Table 1. There were no significant differences in baseline and immediate angiographical measurements among the three groups. At follow-up, late lumen loss in the CB + PEBA group was significantly less than that in the CBA group, but similar to that in the PEBA group ($P > 0.05$).

**Histopathological analyses**

The histological analysis is illustrated in Fig. 1. There were no differences between the three groups in injury score. LA was largest ($P = 0.0043$) but NA and AS% were smallest ($P = 0.0202$ and $P = 0.0015$, respectively) in the CB + PEBA group (Table 2). There were no differences in LA, NA and AS% between the PEBA group and the CB + PEBA group.

Immunohistochemical staining of PCNA is shown in Fig. 2. Levels of protein expression of PCNA were significantly lower in the CB + PEBA group (16.2 ± 4.6%) and the PEBA group (16.1 ± 3.2%) compared with the CBA group (34.7 ± 7.8%, $P = 0.01978$ and $P = 0.0216$, respectively). Quantification of TUNEL staining (Fig. 3) in the intima of the stented arteries showed that TUNEL-positive cells represented 38.1 ± 9.6% of the total number of VSMCs in the CB + PEBA group and 35.9 ± 6.9% in the PEBA group. In contrast, only 16.2 ± 4.7% of VSMCs were TUNEL-positive in the CBA group.

**Table 2: Histomorphometric analysis at follow-up.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>CBA (n = 11)</th>
<th>PEBA (n = 10)</th>
<th>CB + PEBA (n = 10)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>LA (mm²)</td>
<td>2.39 ± 0.77</td>
<td>5.15 ± 0.89*</td>
<td>5.34 ± 1.15*</td>
<td>0.0043</td>
</tr>
<tr>
<td>IELA (mm²)</td>
<td>8.06 ± 0.92</td>
<td>8.12 ± 0.95</td>
<td>8.09 ± 1.62</td>
<td>0.8259</td>
</tr>
<tr>
<td>EELA (mm²)</td>
<td>8.98 ± 0.87</td>
<td>9.10 ± 1.04</td>
<td>9.19 ± 0.91</td>
<td>0.8576</td>
</tr>
<tr>
<td>NA (mm²)</td>
<td>5.68 ± 1.10</td>
<td>2.96 ± 0.67*</td>
<td>2.66 ± 0.69*</td>
<td>0.0202</td>
</tr>
<tr>
<td>AS%</td>
<td>63.36 ± 8.44</td>
<td>37.71 ± 6.31*</td>
<td>34.76 ± 5.83*</td>
<td>0.0015</td>
</tr>
<tr>
<td>Injury score</td>
<td>1.39 ± 0.12</td>
<td>1.36 ± 0.14</td>
<td>1.37 ± 0.14</td>
<td>0.8741</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard deviation. AS%: percentage of lumen area stenosis; CBA: cutting balloon angioplasty; CB + PEBA: cutting balloon followed by paclitaxel-eluting balloon angioplasty; PEBA: paclitaxel-eluting balloon angioplasty; EELA: external elastic lamina area; LA: lumen area; IELA: internal elastic lamina area; NA: neointimal area. A $P$ value < 0.05 indicates a significant difference.

* $P < 0.05$ compared with CBA.

**Figure 2.** Immunohistochemical staining of vascular smooth muscle cells (VSMCs) for proliferating cell nuclear antigen (PCNA) in each group: (A) cutting balloon angioplasty (CBA); (B) paclitaxel-eluting balloon angioplasty (PEBA); (C) cutting balloon followed by paclitaxel-eluting balloon angioplasty (CB + PEBA) ($\times$ 400; bar 50 μm). (D) Percentage of PCNA-positive VSMCs; *$P < 0.05$ compared with CBA.
Discussion

The present study showed that, in a porcine coronary artery restenosis model, CB + PEBA was similar to PEBA alone for prevention of restenosis at 28 days. Furthermore, both CB + PEBA and PEBA were better than CBA alone.

Although drug-eluting stents are currently considered the best possible care in the treatment of ISR [2,3], they are associated with delayed and incomplete endothelialization and an increased risk of stent thrombosis [21–23]. Treatment of patients with ISR remains a challenge and optimal treatment of ISR has not been established.

Previous studies demonstrated that the efficacy of PEBA in the treatment of ISR was similar to that of the drug-eluting stent [16,17,24]. However, it also had some of the shortcomings of conventional balloon angioplasty. The combination of other adjuvant therapies could improve the efficacy and reduce adverse events.

In patients with ISR, intravascular ultrasound studies have shown that CBA most probably acts by first cutting or scoring the neointimal plaque, thus lessening the elastic and fibrotic continuity of the internal fibrous layer and making the tissue more amenable to being pushed outward though the stent struts [25,26]. Compared with conventional angioplasty, CBA is also associated with a decreased incidence of the 'watermelon' or 'soap-bar' effect, which is associated with poorer acute and long-term angiographical results [19]. We assumed that cutting balloon predilatation followed by PEBA for treatment of ISR might better than PEBA alone.

In our study, cutting balloon adjunctive to PEBA did not further reduce late luminal loss and obtain larger LA, compared with PEBA. There are some possible explanations for this unexpected outcome. First, in this animal model of ISR, the preinjured arteries were normal non-atherosclerotic vessels and the nature of their in-stent intimal substrate was composed principally of proliferating VSMCs and extracellular matrix. Whether the information on tissue reaction to cutting balloon in this pig model can be applied to the much more complex ISR in humans is uncertain. Given the structure of the cutting balloon, severe calcified and fibrosis lesions might benefit more.

ISR is caused by neointimal hyperplasia, which involves abnormal growth of VSMCs. Some studies have found that apoptosis may be a beneficial antiproliferative component for the treatment of ISR [27,28]. Our results showed that in the PEBA and CB + PEBA groups, PCNA expression of VSMCs was lower and the percentage of TUNEL-positive VSMCs was higher than in the CBA group. This suggests that PEBA could induce imbalance of apoptosis and proliferation of VSMCs. Accelerating cell apoptosis might be one of the principal mechanisms by which PEBA or CB + PEBA prevent VSMC overproliferation and induce late in-stent neointimal regression.

Study limitations

This study had several limitations, including the small sample size and the inability to perform histological analysis immediately after intervention. The study was also limited to observations in the lesions produced in healthy vessels, the relevance of which to the human clinical condition is uncertain. The observation period was only 4 weeks; this covers the most critical time with respect to thrombotic events and healing, but it remains to be determined for how long the beneficial effect will be maintained in animals.

Figure 3. Terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) staining of each group: (A) cutting balloon angioplasty (CBA); (B) paclitaxel-eluting balloon angioplasty (PEBA); (C) cutting balloon followed by paclitaxel-eluting balloon angioplasty (CB + PEBA). Apoptotic cells are indicated by brown staining of the nucleus. Morphological changes include dense nuclear staining and shrunken cells. However, some positive cells show necrotic morphological changes, such as swelling, membrane rupture and irregular staining of chromatin (×400; bar 100 μm). (D) Percentage of TUNEL-positive vascular smooth muscle cells; *P < 0.05 compared with CBA.
Conclusions

PEBA with or without cutting balloon was superior to CBA for the treatment of ISR in a pig model. The underlying mechanism was probably related to inhibition of VSMC proliferation and increased apoptosis. Treatment of ISR might not require a second stent implantation and PEBA or CB + PEBA seemed to be better.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

References