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Original Research

Drug-Coated Balloons: Drugs Beyond Paclitaxel?

Tobias Haase[1,2,*], Ulrich Speck[1], Stephanie Bienek[2], Melanie Löchel[2], Nadia Brunacci[2], Ole Gemeinhardt[1], Denise Schütt[2], Stephanie Bettink[3], Bettina Kelsch[1,2], Bruno Scheller[3], Beatrix Schnorr[1]

[1]Experimental Radiology, Charité-Universitätsmedizin Berlin, 10117 Berlin, Germany
[2]InnoRa GmbH, 10115 Berlin, Germany
[3]Clinical and Experimental Interventional Cardiology, University of Saarland, 66421 Homburg/Saar, Germany
*Correspondence: tobias.haase@charite.de (Tobias Haase)
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Abstract

Background: Although controversially discussed, paclitaxel is the only clinically proven drug that inhibits restenosis when released from drug-coated balloons (DCBs). Limus drugs are currently being explored as alternatives. The aim of the preclinical studies was to investigate drug candidates beyond paclitaxel considered for balloon coating. **Methods**: Drugs were tested with respect to dissolution in organic solvents, coating on balloons, and drug transfer to the vessel wall. Inhibition of neointimal proliferation was tested in the porcine model of coronary in-stent stenosis. Intravascular drug treatment was achieved by DCBs at the time of stent implantation. **Results**: Coating had to be adjusted for each drug. Doses on the balloons ranged from 1.0 to 8.6 μg/mm^2 balloon surface. Satisfactory amounts of drug ranging from 5% to 29% of initial doses were transferred into the vessel wall. Angiographic parameters such as late lumen loss (LLL) at 4 weeks did not show reduction of in-stent neointimal proliferation by treatment with arsenic trioxide (0.87 $\pm$ 0.44 mm), betamethasone dipropionate (1.00 $\pm$ 0.54 mm), bortezomib (1.74 $\pm$ 0.46 mm), green tea extract (1.24 $\pm$ 0.51 mm), fantolon, an epothilone (0.86 $\pm$ 0.61 mm), methotrexate (1.09 $\pm$ 0.72 mm), and thalidomide (1.59 $\pm$ 0.55 mm) compared to treatment with uncoated balloons (1.07 $\pm$ 0.60 mm), while coatings with paclitaxel reliably reduced in-stent stenosis (LLL = 0.36 $\pm$ 0.25 mm). **Conclusions**: Despite the proven antiproliferative and/or anti-inflammatory effect of the drugs, none of the coatings significantly reduced LLL compared to uncoated balloons and thus, based on the results presented here, none of the tested coatings may be considered a substitute for the paclitaxel-based coatings currently in clinical use.

Keywords: drug coated balloons; in-stent stenosis; neointima formation; paclitaxel; coronary heart disease; endovascular therapies

1. Introduction

Drug-coated balloons (DCBs) are one endovascular treatment option to restore patency of stenotic or occluded coronary and peripheral arteries. While the balloon mechanically reopens or widens the vessel lumen, the drug is delivered into the vessel wall to reduce neointimal proliferation, the main cause of restenosis after balloon angioplasty. Paclitaxel (PTX) was the first drug on DCBs investigated in clinical trials [1] and, to the present day, is the standard drug on DCBs. This might be due to a combination of properties of PTX including fast drug transfer to the vessel wall, strong inhibition of cell proliferation, and long persistence in the vessel wall [2]. On the other hand, the cytostatic activity of PTX and its long tissue persistence might lead to adverse reactions and have raised concerns about its safety. These concerns reinforced the initial research on other drugs [3] in the field of balloon coatings. With its long and successful history in coronary drug-eluting stents (DES), sirolimus has attracted the attention of researchers investigating new DCB technologies. Sirolimus-coated balloons have so far shown promising results in inhibition of restenosis after percutaneous coronary interventions (PCIs) in a small number of patients included in clinical studies [4–8]. Furthermore, a wide range of other drugs with potential antiproliferative, anti-inflammatory, or antithrombotic actions might be candidates for balloon coating. However, due to the short contact time between the balloon and the vessel wall during inflation, pharmacokinetic properties like uptake, distribution, and persistence become especially important. A suitable formulation for a balloon coating requires an adequate amount of a potent drug, proper timing of release at the target site, and uptake of an effective dose into the vessel wall [9]. The aim of the preclinical studies was to test drugs with different modes of action as coatings on angioplasty balloons for inhibition of neointimal proliferation following vessel injury in comparison to uncoated and PTX-coated balloons. Criteria for the selection of a substance were potential antiproliferative and anti-inflammatory activity, commercial availability, and approval for use in humans. Final criteria were applicability as a stable coating on a balloon membrane in a way that facilitates transfer of the drug to the vessel wall. Criteria for the evaluation of efficacy in *in vivo* studies were the angiographic LLL and % in-stent stenosis and the vessel wall area measured histologically in

vessel cross-sections. The following compounds were studied.

1.1 Arsenic Trioxide

Arsenic trioxide (ATO) induces apoptosis and inhibits cell growth and angiogenesis via mechanisms that are not yet fully understood. ATO is available as an intravenously injectable formulation (Trisenox) and is approved for the treatment of acute promyelocytic leukemia [10]. Arsenic trioxide-coated stents have been shown to inhibit neointimal proliferation in preclinical models of in-stent stenosis [11–13].

1.2 Betamethasone Dipropionate (BDP)

Glucocorticoids have an inhibitory effect on inflammatory cell activation, cytokine release, platelet adhesion, smooth muscle cell proliferation, and collagen synthesis [14,15]. In view of these properties, it is tempting to speculate that glucocorticoids might inhibit restenosis after PCIs. Oral glucocorticoid therapy was shown to reduce the occurrence of restenosis after angioplasty [16]. Both preclinical and clinical studies investigating local intravascular administration of glucocorticoids via coated stents found beneficial effects with respect to restenosis inhibition and target lumen revascularization (TLR) [14,17].

1.3 Bortezomib

Bortezomib is a selective proteasome inhibitor that is approved for the treatment of multiple myeloma. It inhibits proliferation and induces apoptosis of metabolically active, rapidly dividing cells at very low concentrations [18]. Systemically and locally delivered bortezomib reduced neointimal proliferation in preclinical models of arterial injury [19,20].

1.4 Epigallocatechin-3-Gallate

Green tea extract contains a number of catechins, including epigallocatechin-3-gallate (EGCG), epigallocatechin (EGC), epicatechin-gallate (ECG), and epicatechin (EC). EGCG was shown to have potential beneficial effects on vascular function via vasodilatory and antiplatelet activity [21–24]. Preclinical studies in rats indicate that EGCG reduces intimal hyperplasia in models of intravascular injury [25,26].

1.5 Epothilones (Fantolon)

Similar to taxol, the epothilones (A and B) are microtubule-stabilizing agents causing cell cycle arrest [27, 28]. Locally administered epothilones suppress neointimal thickening in the rat balloon injury model [29–31]. In our experiments we used fantolon (Bayer-Schering Pharma AG, $C_{30}\,H_{41}\,N\,O_6\,S$) because of its known efficacy at very low dose/concentration.

1.6 Methotrexate

Methotrexate (MTX), an analogue of the B vitamin folic acid, is a first-line synthetic disease-modifying antirheumatic drug for patients with rheumatoid arthritis (RA) and other autoimmune diseases [32]. Systemic (IV) treatment with methotrexate showed beneficial effects in a rabbit model of in-stent neoatherosclerosis [33]. Studies in RA patients at high risk for cardiovascular diseases suggest that MTX has potential protective effects. It might exert beneficial effects on endothelial function, regulating immune responses and inflammation pathways responsible for the development of atherosclerosis and thrombosis [34,35], possibly diminishing in-stent restenosis after PCIs [36].

1.7 Thalidomide

Thalidomide inhibits neoangiogenesis, which contributes to intravascular plaque formation. It is a partial inhibitor of the biosynthesis of the key proinflammatory cytokine tumor necrosis factor alpha (TNFα) [37,38]. TNFα is produced by a number of cells, including macrophages, neutrophils, endothelial cells, and vascular smooth muscle cells (VSMCs) and was shown to play a pivotal role in restenosis [39]. In preclinical models, systemic and local delivery of thalidomide reduced injury-induced neointima formation [40,41].

2. Materials and Methods

All studies presented here were performed between 2007 and 2022 in the same research facilities using standardized protocols as described in previously published reports [3,42,43].

2.1 Balloon Coating

First, we tested the solubility of the selected drugs and their stability in various organic solvents at room temperature. Formulations containing a mixture of the active substance, solvents and, where appropriate, an excipient (Table 1) were coated onto percutaneous transluminal coronary angioplasty (PTCA) balloon catheters (3.5 mm $\times$ 20 mm) by dipping the balloons into the coating solution or by dispersing a precisely defined volume on the balloon surface using a Hamilton syringe (Fig. 1). After complete drying for 24 h at room temperature, coated balloons were visually inspected for appearance, homogeneity, and adherence of the drug coating. The amount of drug on the balloon was analyzed, in most cases by high-pressure liquid chromatography (HPLC) (Table 1).

2.2 In Vivo Evaluation of Balloon Coatings

All *in vivo* experiments were performed at the IMTR (Institute of Medical Technology and Research, Saxony-Anhalt) and conducted in accordance with the guidelines for animal experiments and approved by the animal protection committee of Saxony-Anhalt, Germany. Balloon catheters used for *in vivo* studies were sterilized with ethy-

Table 1. Formulations used for balloon coatings.

Drug		Solvent	Excipient	Analysis
arsenic trioxide		meOH	none	ICP-OES after wet incineration
betamethasone dipropionate		meOH, THF, acetone, H_2O	none	HPLC/UV
bortezomib		ethanol	mannitol	HPLC/UV
EGCG		ethanol, acetone	UV 370	HPLC/UV
fantolon		meOH	PEG 1500	HPLC/UV or MS
methotrexate		meOH, aqueous bicarbonate	none	HPLC/UV
paclitaxel	Paccocath®	ethanol, acetone	UV 370	HPLC/UV
	InPact®	not disclosed	urea	HPLC/UV
	amorphous	dichloromethane	none	HPLC/UV
thalidomide		dioxane, DMFA	NDGA	HPLC/UV

meOH, methanol; THF, tetrahydrofuran; NDGA, nordihydroguaiaretic acid; DMFA, dimethylformamide; PEG, polyethylene glycol; EGCG, epigallocatechin-3-gallate; UV 370, Ultravist™, nonionic, iodinated X-ray contrast agent; ICP-OES, inductively couple plasma optical emission spectrometry; HPLC, high-pressure liquid chromatography; MS, mass spectrometry.

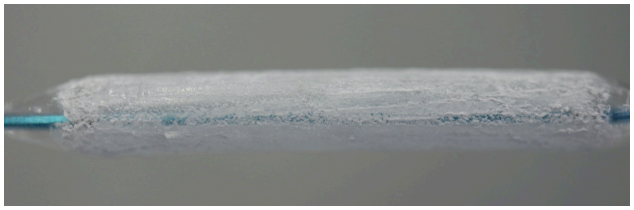

Fig. 1. Drug coated, inflated PCTA balloon.

lene oxide according to a validated protocol. Studies in the porcine coronary in-stent stenosis model were conducted as described in Kelsch *et al.* [42]. Briefly, after sedation, anesthesia and analgesic treatment, the common carotid artery was surgically exposed and an intra-arterial sheath was introduced. The coronary arteries were visualized with a Siemens AXIOM Artis zee fluoroscope using a 6F Judkins L3.5 catheter and Ultravist®-370 as a contrast agent. Coronary angiograms were recorded after intracoronary nitroglycerin administration. After coronary imaging, a suitable segment of the left coronary artery circumflex ramus (CX), left anterior descending artery (LAD), and right coronary artery (RCA) was treated in each pig.

2.3 Drug Transfer to the Vessel Wall

Drug transfer from the balloon surface to the vessel wall was examined as follows: coronary artery segments were labeled with a marker stent. Areas proximal to the implanted marker stent were treated with the coated balloons applying approximately 20% overstretch to ensure vessel wall contact. Balloons were left expanded for 1 minute, deflated, and retracted. The pigs were euthanized immediately after the last treatment in deep anesthesia using supersaturated potassium chloride. The treated vessel segments including the marker stents were dissected for drug analysis.

2.4 Effects on Neointimal Proliferation

Neointimal proliferation was induced by implantation of balloon-expandable bare metal stents using either stents mounted onto balloons (coated or uncoated control) with a hand crimper HH100–101 (Machine Solutions, Flagstaff, AZ) or commercially available premounted bare metal stents on PTCA balloon catheters before postdilatation with experimental DCBs. Stents were expanded in coronary arteries applying approximately 20% overstretch to the vascular segment. Blood flow and lumen dimensions were monitored before and after stent implantation by coronary angiography (see below). Four weeks after the intervention, follow-up angiography of stented segments was done. Then, animals were euthanized in deep anesthesia, and the treated vessel segments were dissected and fixed with 4% buffered formalin for histomorphometric analysis.

2.5 Drug Analysis

Samples containing extraction solvent and unused balloons or balloons after use were intensely shaken on a vortexer for at least 30 seconds, followed by treatment in an ultrasound bath for 30 minutes and centrifugation for 10 minutes. Except for arsenic trioxide, drug concentrations were determined by HPLC with UV detection (Table 2). Drug analysis by HPLC was done on reversed-phase C18 columns (5 μm, 4.6 mm $\times$ 250 mm) or Core-shell columns (2.5 μm, 4.6 mm $\times$ 150 mm). Mobile phases were selected according to the drug. Standards were run together with the samples. For drug extraction from tissues, a defined volume of ethanol was added to achieve an ethanol concentration of $>$50%. Arsenic trioxide was extracted with aqueous NH_4OH. The samples were homogenized (Precelly 24 Dual Homogenizer, PEQLAB Biotechnologie GmbH, Erlangen, Germany) and extracted by 30 minutes ultrasound treatment at room temperature and then centrifuged for 10 minutes at 17,500 $\times$ g. Except for arsenic trioxide, drug concentrations were determined in the supernatant by HPLC with UV detection.

Table 2. Drug content on balloon before and after intervention and transfer to the vessel wall (values given as mean ± SD — if applicable); doses were adjusted according to the known efficacies of the drugs investigated.

Drug	Drug content on balloon	Residual drug on balloon after deployment in animals	Drug in the arterial wall
	(μg/mm^2)	(% of dose on balloon)	(% of dose on balloon*)
arsenic trioxide	3.7	19.6 ± 2.1	10.1 ± 4.8
betamethasone dipropionate	6.0	15.9 ± 4.5	12.9 ± 6.7
bortezomib	1.0	53.0 ± 4.0	not reported
EGCG	8.6	<1	not reported
fantolon	2.4	5.1	5.2
methotrexate	7.6	2.0 ± 2.0	not reported
paclitaxel Paccocath®	3.0	10	29.4 ± 14.7
paclitaxel InPact®	3.5	13	19.7 ± 11.3
paclitaxel amorphous	3.3	30.2 ± 5.12	7.4 ± 6.1
thalidomide	5.9	6.8 ± 3.2	29.0 ± 6.5

* calculated as % of (initial) dose on the balloon measured in the excised vessel segments.

2.6 Quantitative Coronary Angiography

Quantitative coronary angiography (QCA) was done before, during, and after balloon inflation and stent implantation. Four weeks after stent implantation, follow-up QCA was performed at the respective implant sites to assess the primary endpoint late lumen loss (LLL, defined as minimal lumen diameter at implantation (MLD) – minimal lumen diameter at 4-weeks follow-up (MLD FU)) and in-stent stenosis % (defined as (1 – MLD FU / reference diameter (RFD)) × 100) of stented segments. Angiograms were assessed by two experienced observers blinded to the treatment groups using the CAAS II Research System (Pie Medical, The Netherlands) (Fig. 2).

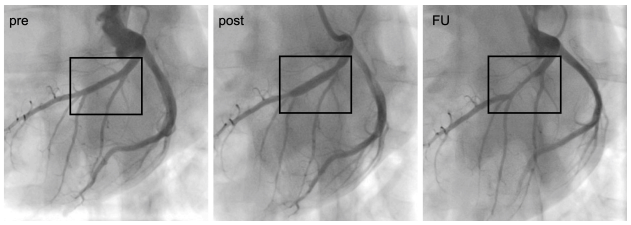

Fig. 2. Angiograms showing a coronary vessel pre- and post treatment with a DCB and at 4-weeks follow-up (FU).

2.7 Histology

The target segments of treated coronary arteries were embedded in methyl-methacrylate, cut with a coping saw, polished, and glued on acrylic plastic slides. Cross-sections from proximal, mid and distal portions of the stent were stained with hematoxylin and eosin (H&E) and/or Masson-Goldner and digitalized. From these images, histomorphometric measurements of lumen and vessel wall area (defined as vessel area – lumen area) were recorded. Injury scores were assigned as described by Schwartz *et al.* [44], and inflammation was graded individually for each stent strut using the score introduced by Kornowski *et al.* [45]. The injury score and the inflammatory score for each cross-section were calculated as the sum of the individual injury and inflammatory scores divided by the number of struts in the examined section.

2.8 Statistical Analysis

QCA-derived parameters were compared across treatment groups. Histomorphometric variables of the three cross-sectional planes were averaged to obtain a mean value per stent. Continuous variables were compared by Kruskal-Wallis one-way ANOVA test using Graphpad Prism (Version 6, GraphPad Software, San Diego, CA, USA). Data are presented as mean ± SD.

3. Results

3.1 Drug Transfer to the Vessel Wall

Compounds were completely dissolved in the aforementioned mixtures and coated on plain 3.5 mm × 20 mm balloons. The results of drug content analysis are compiled in Table 2, given as μg/mm^2 of the total balloon surface (approx. 220 mm^2). Coating composition (solvents and excipients) were selected to accomplish transfer of ≥5% of the dose on the balloon, which corresponds to the lowest percentage of PTX transferred from clinically proven DCBs [42,46].

3.2 Efficacy in Coronary In-Stent Stenosis

On the basis of the *in vitro* and *in vivo* studies, the following drugs were selected to be tested with respect to their efficacy in reducing in-stent stenosis in the porcine coronary overstretch and stent implantation model: arsenic trioxide, betamethasone dipropionate, bortezomib, epigallocatechin-gallate, fantolon, methotrexate, and thalidomide. Both uncoated and PTX-coated balloons, for which inhibition of restenosis is known from controlled clinical trials [47,48], served as controls. Outcome

Table 3. Summary of quantitative coronary angiography results (values given as mean ± SD).

Treatment group	QCA baseline		QCA at 4-week FU		
	RFD (mm, n)	MLD in-stent (mm, n)	MLD in-stent (mm, n)	LLL in-stent (mm, n)	Stenosis in-stent (%, n)
arsenic trioxide	2.51 ± 0.50 (8)	2.78 ± 0.24 (8)	1.91 ± 0.45 (8)	0.87 ± 0.44 (8)	26.8 ± 12.4 (8)
BDP	2.32 ± 0.34 (12)	2.57 ± 0.25 (12)	1.57 ± 0.58 (12)	1.00 ± 0.54 (12)	33.8 ± 19.0 (12)
bortezomib	2.56 ± 0.26 (10)	3.01 ± 0.30 (10)	1.27 ± 0.47 (10)	1.74 ± 0.46 (10)	54.1 ± 14.7 (10)
EGCG	2.39 ± 0.17 (5)	2.38 ± 0.29 (5)	1.13 ± 0.62 (5)	1.24 ± 0.51 (5)	51.8 ± 29.0 (5)
fantolone	2.81 ± 0.30 (10)	2.81 ± 0.27 (10)	2.25 ± 0.52 (10)	0.86 ± 0.61 (10)	20.2 ± 19.3 (10)
methotrexate	2.78 ± 0.42 (38)	3.01 ± 0.30 (38)	1.86 ± 0.68 (38)	1.09 ± 0.72 (38)	32.1 ± 22.8 (38)
thalidomide	2.18 ± 0.40 (10)	2.67 ± 0.17 (10)	1.08 ± 0.54 (10)	1.59 ± 0.55 (10)	53.5 ± 21.9 (10)
uncoated	2.41 ± 0.42 (81)	2.75 ± 0.30 (81)	1.71 ± 0.62 (81)	1.07 ± 0.60 (81)	34.4 ± 20.1 (81)
PTX	2.71 ± 0.37 (48)	2.95 ± 0.23 (48)	2.61 ± 0.22 (48)	0.36 ± 0.25 (48)	6.21 ± 19.3 (48)

n, number of treated arteries; RFD, reference diameter = mean lumen diameter before treatment; MLD, minimal lumen diameter; LLL, late lumen loss = MLD – MLD FU; Stenosis%, (1 – MLD FU / RFD) × 100; BDP, betamethasone dipropionate; EGCG, epigallocatechin-3-gallate; PTX, paclitaxel formulation variants

of balloon treatment in the porcine in-stent stenosis was assessed from *in vivo* coronary angiography and *ex vivo* histological analysis. First, pooled QCA data at 4-week follow-up for uncoated balloons from multiple independent studies were compared with data for the three different PTX coating formulations, namely Paccocath, InPact, and experimental amorphous PTX (see Table 2). All three PTX formulation variants significantly reduced angiographic in-stent LLL compared to the pooled uncoated balloon group (Fig. 3). LLL reduction was not significantly different among the three PTX variants.

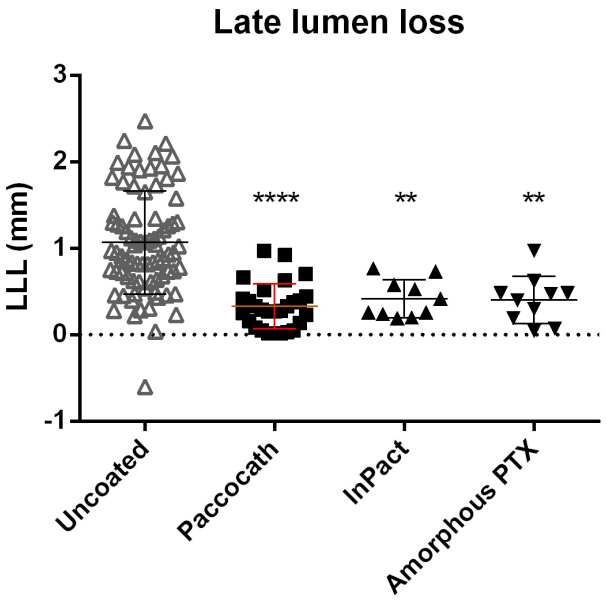

Fig. 3. QCA in-stent late lumen loss (LLL) at 4-week follow-up. Balloons coated with different PTX formulations significantly reduce LLL compared to uncoated balloons. Data are shown as mean ± SD and data points, ****$p \leq 0.0001$, **$p \leq 0.01$ vs. uncoated control, Kruskal-Wallis test with Dunn's post hoc comparison.

To elucidate a potential inhibition of neointimal proliferation of the different novel balloon coatings, we compared data points from multiple experiments investigating the drug candidates with the pooled results achieved with uncoated control balloons and PTX formulation variants. For all studies, quantitative coronary angiography revealed similar angiographic baseline parameters in each treatment group within each study versus the uncoated control. Except for treatment with PTX-coated balloons, angiographic parameters at 4 weeks after implantation did not show a significant reduction of in-stent stenosis in any of the tested drug-coated balloon groups compared to uncoated controls (Table 3, Fig. 4). Unlike treatment with PTX-coated balloons, none of the new coatings we investigated inhibited neointimal proliferation, i.e., QCA showed no decrease in LLL or in-stent stenosis or increase in minimal lumen diameter compared to treatment with uncoated balloons.

3.3 Histological Analysis

Histological analysis of stented cross-sections showed no reduction of neointimal area for any balloon coatings except PTX compared to the uncoated control group (Table 4, Fig. 5). These results confirm the ineffectiveness of the tested balloon coatings with respect to inhibition of neointimal proliferation.

Cross-sections of stented artery segments showed significant neointimal thickening and lumen narrowing for all balloon coatings investigated except for the PTX group. Nearly all sections from arteries treated with PTX-coated balloons exhibited only a thin cellular layer covering the stent struts (Fig. 6).

3.4 Tolerance

None of the tested devices, coatings, or applications caused any signs of obvious toxicity during the experiments. None of the drugs had to be excluded from further testing because of recognizable local or systemic toxicity.

Table 4. Summary of histomorphometry results at 4-week follow-up (values given as mean ± SD).

Treatment group	Lumen area (mm^2, n)	Vessel wall area (mm^2, n)	Injury score (n)	Inflammation score (n)
arsenic trioxide	4.27 ± 1.84 (8)	4.09 ± 0.75 (8)	2.53 ± 0.38 (8)	2.64 ± 0.40 (8)
BDP	3.52 ± 1.65 (12)	4.70 ± 1.63 (12)	1.67 ± 0.69 (12)	2.03 ± 0.58 (12)
bortezomib	4.12 ± 1.52 (10)	5.76 ± 1.48 (10)	1.75 ± 0.66 (10)	2.05 ± 0.84 (10)
EGCG	2.04 ± 0.96 (5)	5.39 ± 1.92 (5)	2.14 (1)	3.00 (1)
fantolon	not done	not done	not done	not done
methotrexate	3.66 ± 1.08 (18)	4.40 ± 1.82 (18)	1.27 ± 0.70 (18)	1.56 ± 0.78 (18)
thalidomide	4.34 ± 0.36 (2)	4.67 ± 0.04 (2)	not done	not done
uncoated	4.12 ± 1.85 (81)	5.03 ± 1.55 (81)	1.94 ± 0.79 (76)	2.04 ± 0.94 (76)
PTX	5.86 ± 1.47 (50)	2.92 ± 1.09 (50)	1.75 ± 0.79 (48)	2.30 ± 0.82 (48)

n, number of treated arteries available for evaluation; BDP, betamethasone dipropionate; EGCG, epigallocatechin-3-gallate; PTX, paclitaxel formulation variants.

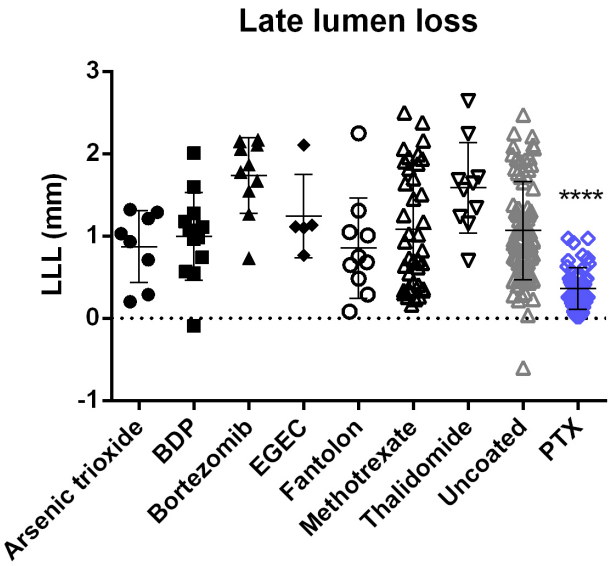

Fig. 4. QCA in-stent late lumen loss (LLL) at 4-week follow-up. Following treatment with PTX-coated balloons, LLL is significantly lower compared with the pooled results achieved with uncoated balloons. For all other coatings tested, results are not significantly different from those of the uncoated control group. Data are shown as mean ± SD and data points, ****$p \leq 0.0001$ vs. uncoated control, Kruskal-Wallis test with Dunn's post hoc comparison.

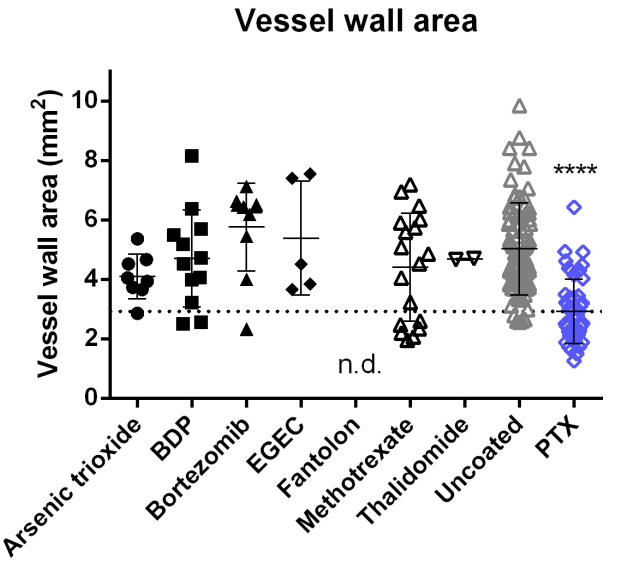

Fig. 5. Histomorphometric in-stent vessel wall area at 4-week follow-up. Following treatment with PTX-coated balloons, the vessel wall area (including neointima) is significantly smaller than after treatment with uncoated balloons. For all other coatings tested, results are not significantly different from those of the uncoated control group. Data are shown as mean ± SD and data points, PTX mean – dotted line, ****$p \leq 0.0001$ vs. uncoated control, Kruskal-Wallis test with Dunn's post hoc comparison.

4. Discussion

Despite the antiproliferative and/or anti-inflammatory effects of the drugs in the coatings investigated here (for details please see introduction), the results of our experiments were disappointing. The coating formulations were adjusted to accomplish a transfer similar to that published for paclitaxel (i.e., ≥5% of dose on balloons [2,46]). Several drugs required an additive to modulate adherence and release, e.g., thalidomide plus NDGA, but other drugs showed respectable acute transfer into the vessel wall without an excipient, e.g., betamethasone dipropionate. Some of the coating formulations we developed did not transfer sufficient amounts of the active agent into the vessel wall during balloon inflation and were excluded from further testing.

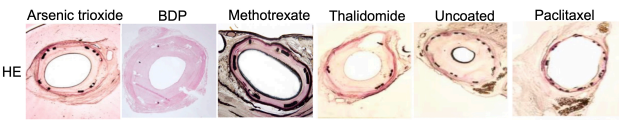

Fig. 6. Representative H&E-stained histological cross-sections of stented artery segments at 4-week follow-up. BDP, betamethasone dipropionate.

While LLL following treatment with PTX-coated balloons was consistently low in our studies (with means of

individual studies ranging from 0.22 to 0.42 mm LLL; see Table 3; Figs. 3,4), all investigated non-paclitaxel formulations showed no effect in terms of inhibition of neointima formation compared to uncoated balloons despite acute transfer of a respectable percentage of the dose into the vessel wall (e.g., thalidomide coating: about 30% of dose recovered in the arterial wall and LLL of 1.59 $\pm$ 0.55 mm vs 1.44 $\pm$ 0.62 mm for uncoated balloons, same experiment, n = 10 vs. n = 12), indicating that the drug is rapidly metabolized or eliminated or simply does not inhibit neointimal proliferation in this model. The absent or at best very moderate efficacy in the current studies may be attributable to several factors including mode of action of the drug, the use of inappropriate volatile solvent or matrix/excipient compositions for coating, insufficient dose on the balloons or at the target site, respectively, or an inappropriate balloon surface for the selected drug. Drug transfer to the vessel wall could be modulated by altering drug formulation (e.g., crystals, particles) or using devices (e.g., with spikes [49]) that promote transfer into the tissue.

In our experiments, dichloromethane formulation yielded an amorphus paclitaxel coating that showed an inhibition in in-stent stenosis similar to other paclitaxel coatings. This is in contrast to the amorphus paclitaxel-iopromide coating of Granada *et al*. [50], which seemed to be ineffective in the porcine in-stent stenosis model. Even for PTX-coated balloons, it has long been known that there is no class effect and that inhibition of neointimal proliferation depends critically on the coating composition and method rather than the active drug alone [46,51].

Although paclitaxel coated balloons were developed in the porcine in-stent stenosis model, it may not be the appropriate model for testing these novel drugs. In porcine studies, a significantly stronger reduction of neointima formation by paclitaxel compared with sirolimus was seen [43]. These findings are in contrast to coronary clinical data where Limus stents exhibit equivalent restenosis inhibition and dominate the market. The differences may be related to the fact that the animal model (juvenile animals, no atherosclerosis, and artificial overstretch) does not fully reflect the situation in humans. Furthermore, different biological responses may be postulated for pigs and humans [3].

Our current study shows that the findings for paclitaxel coatings do not seem to be easily transferable to other agents and give an insight into the difficulties of developing new drug coatings.

5. Conclusions

Also, in response to frequent criticism of PTX, we systematically investigated several drugs to explore their potential use as balloon coatings for inhibiting neointimal proliferation in the animal model which led to the discovery of clinically proven DCBs. Despite potent antiproliferative effects of some drugs and successful drug transfer into the vessel wall, their inhibitory effect on neointimal formation in porcine coronary arteries was far below that of PTX-coatings in our experiments. Reasons for the lacking efficacy may be the solvent compositions and/or additive used, the balloon surface, and the animal model. Better outcomes may be achieved with other coating formulations and/or different balloons.

Abbreviations

BDP, betamethasone dipropionate; EGCG, Epigallocatechin-3-gallate; LLL, late lumen loss; MLD, minimal lumen diameter; PTX, paclitaxel; RFD, reference diameter; QCA, quantitative coronary angiography.

Author Contributions

TH, US, BSchn designed the research study. TH, SBie, ML, NB, OG, DS, SBet, BK, BSche, BSchn performed the research and analyzed the data. TH, US, BSchn wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

The animal experiments were approved by the local animal ethics committee (Landesverwaltungsamt LVwA Saxony-Anhalt, Germany) with the approval number (42502-2-1508) and were conducted in accordance with European commission directive 86/609/EEC and the German Animal Protection Act.

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Conflict of Interest

US and BSche are shareholder of InnoRa GmbH. TH, SBie, ML, NB, DS, BK are employees of InnoRa.

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