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Acute drug transfer and particle release of drug coated balloons within a porcine in-vitro model

Abstract: Drug coated balloons (DCB) are used in the therapy of coronary as well as peripheral artery disease. The success of drug transfer to the vessel wall depends on the excipient used in combination with paclitaxel as antiproliferative drug. Although in-vivo studies show very good results with this technology, there is a lack of in-vitro test methods for characterization of various DCB available on the market. This study describes a method to gain information about the drug transfer and the particle release of three different DCB based on cetylpyridinium salycate (Cetpyrsal), hyaluronic acid and iopromide within a porcine in-vitro model. The Cetpyrsal-based DCB showed promising results with the highest drug transfer while producing the lowest number of particles.

Keywords: Drug coated balloon, drug transfer, in-vitro, Paclitaxel, particulate evaluation.

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1 Introduction

Drug-coated balloons (DCB) represent an alternative treatment option for arteriosclerotic vascular disease [1]. A balloon providing an antiproliferative drug, e.g. paclitaxel (PTX), is dilated into the narrowed vessel. In addition to plain angioplasty, PTX is transferred to the vessel wall to prevent restenosis by inhibition of smooth muscle cells [2]. Typically, PTX is embedded in a certain carrier substance or excipient. After the first proof of concept in 2006 by Scheller et al. with a PTX-iopromide DCB, several other excipients such as shellac, urea, polysorbate/sorbitol, citric acid esters, polyethylene glycol or n-butyryl tri-n-hexyl citrate in combination with PTX are in clinical use [1, 3, 4]. Clinical studies showed very promising results in the therapy of coronary as well as peripheral artery disease, especially for treatment of in-stent restenosis [1].

To reduce the drug loss during advancing the DCB to the final lesion and to improve drug transfer to the vessel wall, new drug coatings based on cetylpyridinium salycate (Cetpyrsal) or hyaluronic acid (HA) have been developed and tested in an in-vitro track model [5, 6]. This model is limited in a way that artificial materials like Teflon or silicone are used to mimic the arterial walls in-vitro. Thus, it is difficult to predict the actual drug transfer to the vessel in-vivo.

This study describes a method to gain information about the in-vivo drug transfer in a simple porcine in-vitro model, comparing a commercial available DCB with two alternative coatings developed by Petersen et al. [5, 6].

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2 Materials and methods

Conventional semi-compliant balloon catheters of the size 3.0/20 mm (BIOTRONIK SE & Co. KG, Bülach, Switzerland) were manually coated by a Cetpyrsal-based coating or a HA-based coating, respectively.

The ionic liquid Cetpyrsal was synthesized from cetylpyridinium chloride and sodium salicylate [5]. The Cetpyrsal-based coating was produced as described by Kaule et al. [7]: PTX and Cetpyrsal were dissolved separately in methanol. The PTX solution was diluted 1:1 with the Cetpyrsal solution to obtain a PTX concentration of 50 % (w)

in Cetpyrsal. Finally, 100 µl of the Cetpyrsal-PTX-coating solution was pipetted on the balloon catheter.

The HA-based coating was manufactured according to Petersen et al. [6]. After chemical attachment of the HA base layer further HA layers were applied by manually dip coating into a HA-solution and into a glutardialdehyde solution for crosslinking. The dip coating process was repeated ten times. The PTX incorporation was performed via pipetting 100 µl of the coating solution (PTX dissolved in ethanol/H_2O at a ratio of 8/2 (v/v)) on the balloon catheter.

As reference commercially available DCB catheters with a PTX-iopromide coating (SeQuent Please, B. Braun Melsungen AG, Berlin, Germany) of the size 3.0/20 mm were used.

Porcine carotid arteries with an inner diameter of 3.0 mm and a length of 40 mm served as porcine in-vitro model. The inner diameter was verified by calibrated pin gauges (Knuth Werkzeugmaschinen GmbH, Wasbek, Germany) of 0.1 mm steps in diameter.

The in-vitro drug transfer test was performed as follows: The porcine carotid artery was immersed into a vial with 40 ml of 0.9 % saline solution and pre-warmed up to 37 °C. Then the balloon was inserted into the artery and dilated up to a diameter of 3.3 mm (10 % over-size). The pressure was held for 30 s. Afterwards the balloon was deflated and removed from the artery and the vial. The balloon was cut off and immersed into 30 ml methanol to obtain the remaining drug amount on the balloon. The carotid artery was subsequently removed from the saline solution and immersed into another vial with methanol to obtain the acute amount of drug transferred to the vessel wall. The vial with saline solution was used for particle measurements as well as for obtaining the drug amount, lost during the procedure (see **Figure 1**).

The amount of PTX in all test solutions was determined by high-performance liquid chromatography (HPLC). Therefore all methanol and saline solutions were diluted with methanol 1:10 (v/v) or 1:1 (v/v), respectively.

For HPLC analysis 20 µl of each aliquot were injected into an Eurospher 100-5 C18 column, 120 mm x 4 mm ID. The chromatographic conditions were: column temperature 23 °C; isocratic eluent PBS (0.005 M, pH 3.5) - acetonitrile 50 – 50 % (v/v), flow rate 1.0 mL/min and UV detection at 230 nm with a detection limit of approximately 0.12 µg/mL.

Sub-visible particles were analyzed according to USP 788 [8] using a HIAC ROYCO 9703 Liquid Particle Counting System (sensor model HRLD400, HACH, Loveland, Colorado, USA). Before starting the measurements all liquid containers were degassed for one hour and carefully inverted 20 times for homogenization of the particle distribution. Assessment of particle numbers was performed for particle size classes ≥ 10 µm, ≥ 25 µm and ≥ 50 µm.

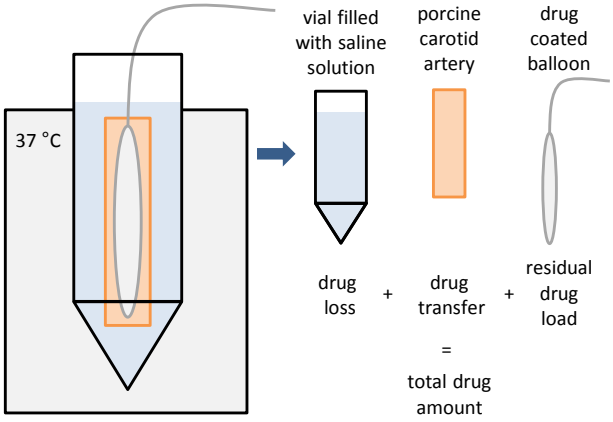

Figure 1: Schematic representation of the in-vitro drug transfer test

3 Results

The measured PTX amounts (drug loss, drug transfer and residual drug load) are given as absolute value, as amount per balloon area (A = 188.5 mm² for the used 3.0/20 mm balloons) as well as relative drug amount (see **Table 1**).

Table 1: Drug loss, transfer and residual drug load of the different coated DCB during the in vitro drug transfer test (SDL = systemic drug loss, ADT = acute drug transfer to the vessel wall, RDL = residual drug load on the balloon after deflation and removal, TDL = totalized drug load)

		Cetpyrsal-based DCB 3.0/20 mm (n = 5)	HA-based DCB 3.0/20 mm (n = 6)	SeQuent Please DCB 3.0/20 mm (n= 5)
SDL	[µg]	26.7 ± 21.2	52.6 ± 24.5	93.3 ± 37.7
	[µg/mm²]	0.14 ± 0.11	0.28 ± 0.13	0.50 ± 0.20
	[%]	6.2 ± 5.0	12.7 ± 5.8	17.3 ± 7.4
ADT	[µg]	349.7 ± 87.4	231.6 ± 52.8	389.1 ± 66.4
	[µg/mm²]	1.86 ± 0.46	1.23 ± 0.28	2.06 ± 0.35
	[%]	76.6 ± 8.2	55.8 ± 11.8	70.6 ± 8.2
RDL	[µg]	76.9 ± 26.9	129.6 ± 48.8	67.0 ± 21.9
	[µg/mm²]	0.41 ± 0.14	0.69 ± 0.26	0.36 ± 0.12
	[%]	17.2 ± 5.6	31.5 ± 12.4	12.1 ± 3.0
TDL	[µg]	453.3 ± 83.4	413.7 ± 14.7	549.3 ± 51.8
	[µg/mm²]	2.40 ± 0.44	2.20 ± 0.08	2.91 ± 0.27

The totalized drug loads result from the sum of drug loss, drug transfer and residual drug load on the balloon. Contrary to the commercially available SeQuent Please DCB the total drug found was found lower than expected for the Cetpyrsal-based DCB and the HA-based DCB (2.4 µg/mm² and 2.2 µg/mm², respectively). The relative drug loss, relative

drug transfer and relative residual drug load were therefore related to the totalized drug load of each test sample.
The acute drug transfer was highest for the Cetpyrsal-based DCB (77 %), followed by the SeQuent Please DCB (71 %) and smallest for the HA-based DCB (56 %). The drug loss during the procedure was highest for the SeQuent Please (17 %) and lowest for the Cetpyrsal-based DCB (6 %). The residual drug load on the balloon after the procedure was 31 % for the HA-based DCB, 17 % for the Cetpyrsal-based DCB and 12 % for the SeQuent Please (see **Figure 2**).

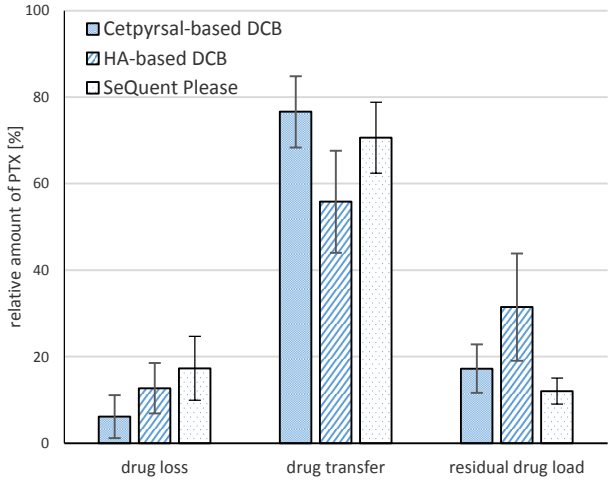

Figure 2: Results of the in-vitro drug transfer test – relative amount of PTX related to the totalized drug load

The numbers of particles that were released into the saline solution during the in-vitro drug transfer test are presented in **Table 2**.

Table 2: Number of particles released from the different DCB during the in-vitro drug transfer test

Particle size	Cetpyrsal-based DCB 3.0/20 mm (n = 5)	HA-based DCB 3.0/20 mm (n = 6)	SeQuent Please DCB 3.0/20 mm (n= 5)
≥ 10 µm	20,873 ± 15,360	105,899 ± 36,735	63,640 ± 15,038
≥ 25 µm	1,810 ± 1,469	2,144 ± 892	5,800 ± 1,100
≥ 50 µm	325 ± 357	217 ± 166	848 ± 202

Particle release of particles ≥ 10 µm was highest for the HA-based DCB (106,000 ± 37,000), followed by SeQuent Please (64,000 ± 15,000) and the Cetpyrsal-based DCB (21,000 ± 15,000). Considering particles ≥ 25 µm and ≥ 50 µm SeQuent Please showed the highest particle numbers (5,800 ± 1,100 and 850 ± 200 particles, respectively). The Cetpyrsal- and HA-based DCB showed similar results for particles ≥ 25 µm (1,800 ± 1,500 and 2,100 ± 900 particles, respectively) as well as for particles ≥ 50 µm (330 ± 360 and 220 ± 170 particles, respectively).

4 Discussion

An effective coating is necessary to achieve an efficient transfer of PTX from the balloon to the arterial wall [9]. The in-vivo drug transfer is reported in literature between 2 % and 20 % of the initial drug load, depending on the animal model and the specific DCB used [2, 10-12].

Several studies dealt with the investigation of the drug transfer as well as particle release of different DCB in-vitro [5-7, 13-15]. In-vitro drug transfer into a silicone mock vessel after the passage of a Cetpyrsal-based DCB through a track model is reported with 40 % of the initial balloon drug dose [5, 7, 14]. If results determined by Kaule et al. are recalculated by referring the drug transfer to the initial load minus the drug loss during track, the recalculated drug transfer into the silicone tube was 55 % for the Cetpyrsal- and 28 % for the HA-based DCB [7]. The drug transfer as determined with our porcine in-vitro model was 1.4 times or 2 times higher, respectively. In contrast, the reported acute drug transfer into a silicone tube for the HA-based DCB is 50 % of the initial drug load of the balloon [6], which is in good agreement with our results. Kempin et al. showed a good correlation of drug transfer for the SeQuent Please DCB after tracking through a vessel model into an alginate film as well as a carotid artery (17.7 % vs. 17.4 %). The drug transfer into the alginate film without previous tracking was almost three-fold (48 %) [13], but still lower than our data (71 %). Even if drug transfer was much higher for all in-vitro tests than the reported in-vivo data it is assumed that a porcine vessel model represents a good alternative as the inner vessel surface reflects most likely the in-vivo situation.

Babcock et al. measured 15,000 to 73,000 particles ≥ 10 µm per catheter for different coated angioplasty catheters after tracking through an in-vitro track model, dilatation into a silicone tube and retraction from the model [15]. Our results are similar, but only considering the inflation/deflation process. It is therefore assumed that the overall number of particles would be even higher, if the DCB were additionally tracked through an in-vitro model. This thesis is supported by particle measurements by Kaule et al., that show 1.4 to 10.5 times higher number of particles ≥ 10 µm for Cetpyrsal-based DCB and HA-based DCB, respectively, were generated after track compared to the particles generated during the dilatation process [7].

However, our measurements give indication of the general integrity of the coating and the size distribution of the particles generated for a first rating of different coatings.

5 Summary and conclusion

In this study we presented a simple in-vitro setup to gain information about the acute drug transfer and particle release of DCB in-vivo using a Cetpyrsal- and HA-based DCB as well as the SeQuent Please DCB as representative test samples. The Cetpyrsal-based DCB showed promising results with the highest drug transfer, while producing the lowest number of particles. As the in-vivo drug transfer is reported much lower than determined in our study and with other artificial vessel models, further investigation is needed to mimic the in-vivo situation of a drug coated balloon application in-vitro.

Author's Statement

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