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Research article

Local delivery of paclitaxel for the prophylaxis of restenosis after experimental balloon dilatation

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Abstract:

Introduction: Paclitaxel is an agent with potent antitumor activity that has been approved for clinical use in patients with different types of cancers. It influences the cytoskeleton equilibrium by increasing the assembly of altered microtubules. The aim of this study was to evaluate the safety and efficacy of local delivered paclitaxel after experimental balloon dilatation.

Material and methods: Seventeen domestic pigs underwent balloon dilatation of the proximal left anterior descending artery. In the treatment group (n=8) paclitaxel (10 ml; 10 micromole/L) was delivered using the double-balloon perfusion catheter. The control group (n=9) received only physiological saline under the same circumstances. The animals were sacrificed 4 weeks later. Vessels were perfusion-fixed and histomorphometric analysis was performed using conventional techniques.

Results: The vessel lumen of the treatment group was significantly larger (2.13±1.2 mm^2) than that of the control group (1.38±0.6mm^2) ($p<0.05$). The degree of stenosis in the treatment group was significantly lower (22.2±13.2%) than that of the control group (53.7±21.5%) ($p<0.05$). The intimal area of the treatment group was significantly lower (0.61±0.5mm^2) than that of the control group (1.78±1.0mm^2)

Conclusion: The favorable features of paclitaxel for local application ant the advantageous mode of action suggest that this drug seems to be effective and safe for the prophylaxis of restenosis.

Keywords: Paclitaxel, local drug delivery, coronary restenosis

Introduction

The antineoplastic compound paclitaxel causes an increased assembly of extraordinarily stable microtubules.[1] Further, it reduces many cellular functions like proliferation, migration, and signal transduction.[2, 3] Restenosis after coronary angioplasty is due to a complex cascade of fibroproliferative reactions to arterial wall injury involving vascular smooth muscle cell migration and proliferation with neointimal accumulation.[4] Paclitaxel was found to interfere with SMC migration and proliferation in rat and rabbit model studies involving the carotid artery.[4, 5] This makes paclitaxel a very promising candidate for local drug therapy to address the migratory and proliferative responses involved in restenosis following percutaneous coronary intervention.[6]

The current study was designed to evaluate the effects of locally delivered paclitaxel in an experimental porcine angioplasty model. For the local drug therapy a double-balloon perfusion catheter was chosen because its use enables local delivery of especially lipophilic substances and additional vascular injury is avoided in comparison to the use of active injection catheters. Further, local drug therapy was preferred due to immense systemic side effects of paclitaxel in case of a systemic application.[7]

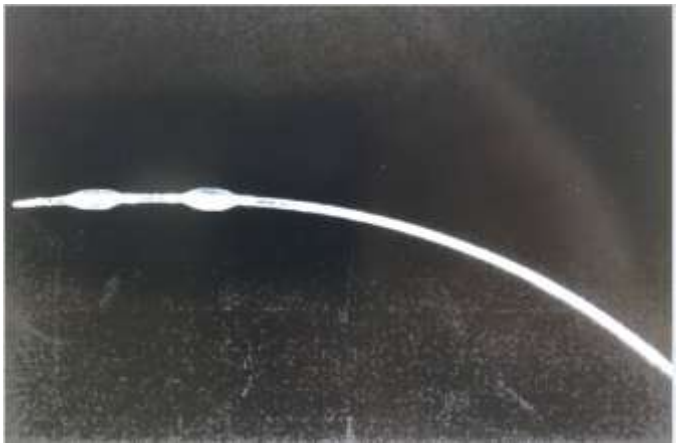
Material and methods

Paclitaxel

Paclitaxel (Sigma, Deisenhofen, Germany) is extracted from the bark of the yew tree Taxus brevifolia. Chemically it is of highly lipophilic nature. A concentration of 10 µmole/L was used, which is the same concentration shown to be effective for inhibition of smooth muscle cell proliferation in in vitro and in vivo studies.[1, 5]

Double-Balloon Perfusion Catheter

The device (Schneider Europe, Bülach, Switzerland) is a 3French 4-lumen polyethylene catheter having a proximal infusion port, main catheter shaft, and a distal infusion compound with a proximal 3.5 mm compliant urethane balloon and a distal 3.1 mm balloon (Figure 1).



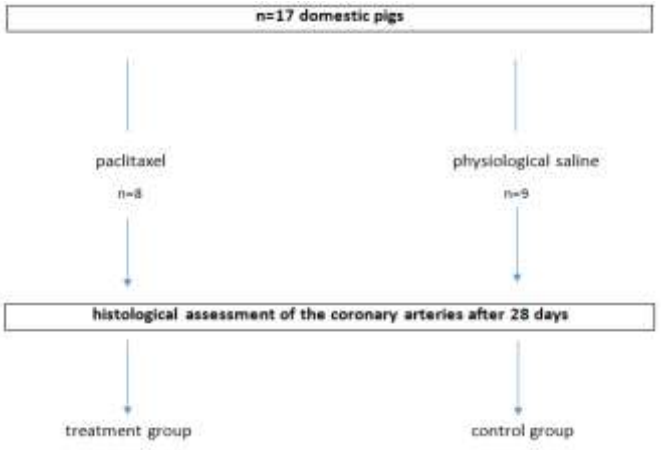
The distance between those is 2.0 cm. Paclitaxel is administered via the proximal infusion port and is delivered to the infusion region. The balloons were inflated to 3 atmosphere (atm). A flow of 50-60 ml/min can be achieved through the perfusion lumen.

Animal Model

The intervention was performed on 17 domestic pigs (weight, 24-27 kg) under general anesthesia. The study protocol was approved by the University of Tübingen, Germany ethical committee. The investigation conforms to the Guide for Care of Use of Laboratory Animals published by the U.S. National Institutes of Health.

The premedication of the animals was performed with aspirin per os (750 mg) 24 hours before the procedure. A single bolus dose of heparin (7.500 U) was given intravenously. All pigs were sedated, intubated, and ventilated with isoflurane with a mixture of 50% O_2 and 50% N_2O at a rate of 30/ml/kg/min. The carotid artery was used as the access site. We inserted an arterial hemostatic sheath (8 French). A conventional percutaneous coronary intervention guide was advanced to the aortic root and placed under fluoroscopy at the ostium of the left main coronary artery (LAD). A floppy guide wire was advanced to the distal and of the LAD. A balloon angioplasty (diameter of 3.5 mm and length of 20 mm) was performed at the mid portion of the vessel as the target site under fluoroscopy (Figure 2).

The inflation pressure was 6 atm. Then the Double-Balloon catheter was placed at the dilatation site. Via a handheld syringe with pressure monitoring, 10 ml drug was given. The duration of drug application was 3 minutes. The animals randomized to control group received a physiological saline infusion under the same circumstances (Figure 3).



Then the sheath was removed and the carotid incision site ligated. Pigs were returned to routine care, including daily monitoring.

All animals were sacrificed 28 days after the intervention using an intravenous euthanasia solution. The heart was removed immediately and perfusion-fixed for 24 hours (10% buffered formalin). The coronary artery segments with intervention were removed and sectioned. These segments were embedded in paraffin and stained with hematoxylin and eosin and elastic van Gieson stains. Embedded vessels were cut were cut into cross-sections beginning at the caudal end of the intervened region. Approximately 10-12 cross-sections (4µm thick) were assessed and used for histological analysis.

The sections were studied qualitatively by light microscopy. Quantitative analysis was performed digital planimetry system for length and area measurements (Bilaney Consulting, Düsseldorf, Germany). Quantitative measurement of the degree of proliferation was performed. The lumen area, degree of stenosis, and the intima and media area were calculated (Figure 4).

Immunohistochemistry

Alpha-actin staining (Anti-SM-α-Actin, Sigma Chemical, St. Louis, MO) was performed to confirm the origin of the smooth muscle cells.

Statistical analysis

The study was designed to compare the response to injury of the control group compared to animals receiving paclitaxel via local delivery. Continuous variables are described by use of statistical characteristics (means, standard deviations). The results of the morphometric analysis were compared with the Wilcoxon test between the two groups. P values <0.05) were considered to identify significant differences).

Results

The vessel lumen of the treatment group was significantly larger (2.13±1.2 mm^2) than that of the control group (1.38±0.6mm^2) ($p<0.05$). The degree of stenosis in the treatment group was significantly lower (22.2±13.2%) than that of the control group (53.7±21.5%) ($p<0.05$). The intimal area of the treatment group was significantly smaller (0.61±0.5mm^2) than that of the control group (1.78±1.0mm^2). The area of the media layer in the treatment group was 1.5±0.5

mm^2. The control group had a media layer area of 1.9±0.5 mm^2. There was no statistical significant difference (Table 1).

Table 1: Results of the histological assessment 28 days after local drug delivery of the LAD

Variables	Treatment group (n=8)	Control group (n=9)	P value
Vessel lumen (mm^2)	2.13±1.2	1.38±0.6	<0.05
Stenosis (%)	22.2±13.2	53.7±21.5	<0.05
Intima area (mm^2)	0.61±0.5	1.75±1.0	<0.05
Media area (mm^2)	1.5±0.5	1.9±0.5	Non-significant

Discussion

In this study we aimed to assess the efficacy and safety of paclitaxel which was delivered locally into the coronary artery of the pig model. This agent has shown a significant antineoplastic impact in various human tumors like ovarian, breast, head and neck cancer.[8] The mode of action of paclitaxel is decreasing the critical concentration of tubulin required for microtubule assembly in the cell.[9] It is highly lipophilic, which promotes a rapid cellular uptake, and has a long-lasting effect in the cell due to structural alteration of the cytoskeleton.[10, 11] But the use of paclitaxel in context with restenosis was first described by Sollot et al.[4] Restenosis after percutaneous balloon angioplasty remains still an unsolved problem and is considered as the 'Achilles tendon' of percutaneous coronary intervention.[12, 13] The pathophysiology of restenosis is still not fully elucidated.[14, 15] Our current understanding suggests that it is a multifactorial process.[16, 17] The lesion (induced by angioplasty) of the arterial wall triggers a cascade of reactions with release of growth factors like platelet-derived growth factor (GF), epidermal GF, and insulin-like GF.[18,19] These lead to transformation of the SMC of the media from a contractile to a synthesizing phenotype with proliferation and migration into the intima.[20] Further, synthesis and secretion of extracellular matrix ensues with consequent progress of restenosis.[21] Paclitaxel in this context inhibited SMC migration and proliferation in rat and human cell cultures.[22, 23] This was shown by a study of Axel et al.: They used monocultures of human arterial SMC and cocultures with human arterial endothelial cells. The growth of the cells was assessed in the presence or absence of platelet derived GF. Single dose application or nonstop exposure of paclitaxel caused inhibition of proliferation of human arterial SMC.[1] Further studies with the rat and rabbit model showed similar results: Sollot et al. demonstrated in the rat model that taxol prevented arterial SMC proliferation and the neointimal arterial SMC accumulation after balloon angioplasty and endothelial injury of the carotid artery.[4] In another study the carotid artery of the rabbit model was dilated with a balloon catheter leading to an intimal injury. In the treatment group, paclitaxel was given with a microporous balloon catheter to the injury site. Compared with the control group not receiving paclitaxel, the treatment group had a statistically reduced degree of stenosis of the carotid artery.[24]

Taking these positive results into consideration, our hypothesis was to test the drug in the porcine model. The pig model is of advantage because the physiology of the human cardiovascular system has more similarity with the porcine than the dog or the rabbit model.[25] Further, the size of the porcine coronary arteries is comparable with the human coronaries and allows the use of human coronary catheters.[26] Also the anatomy and the collateral vessels of the pig model are very similar to those of the humans.[27] Another advantage of the pig model is the uncomplicated induction of pathological situations like atherosclerosis, arterial hypertension and diabetes mellitus. Finally, the histological composition of the atherosclerotic lesion in the pig model is better comparable with the human atherosclerosis than that in the rabbit model.[26]

Our results show that paclitaxel application using the double-balloon perfusion catheter reduced neointima formation in the coronary artery of the pigs. We could demonstrate that the treatment group had a larger vessel lumen, reduced degree of stenosis and also a significantly smaller intimal area. These findings are in accordance with many studies conducted in this field.[4, 5]

Lastly the advantages of local drug delivery with the double-balloon catheter have to be mentioned. By this technique, the drug can be applied in high and constant doses only at the target site. Further, possible systemic intoxication or side effects can be minimized.[28] The insertion site of this catheter into the body is also very small and local anesthesia only is needed. Consequently, complications like bleeding infection or anesthetic accident are very low.

Conclusion

According to the results of our study, we think the favorable features of paclitaxel and its advantageous mode of action may be effective and safe for the local drug delivery to prevent coronary artery restenosis.

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