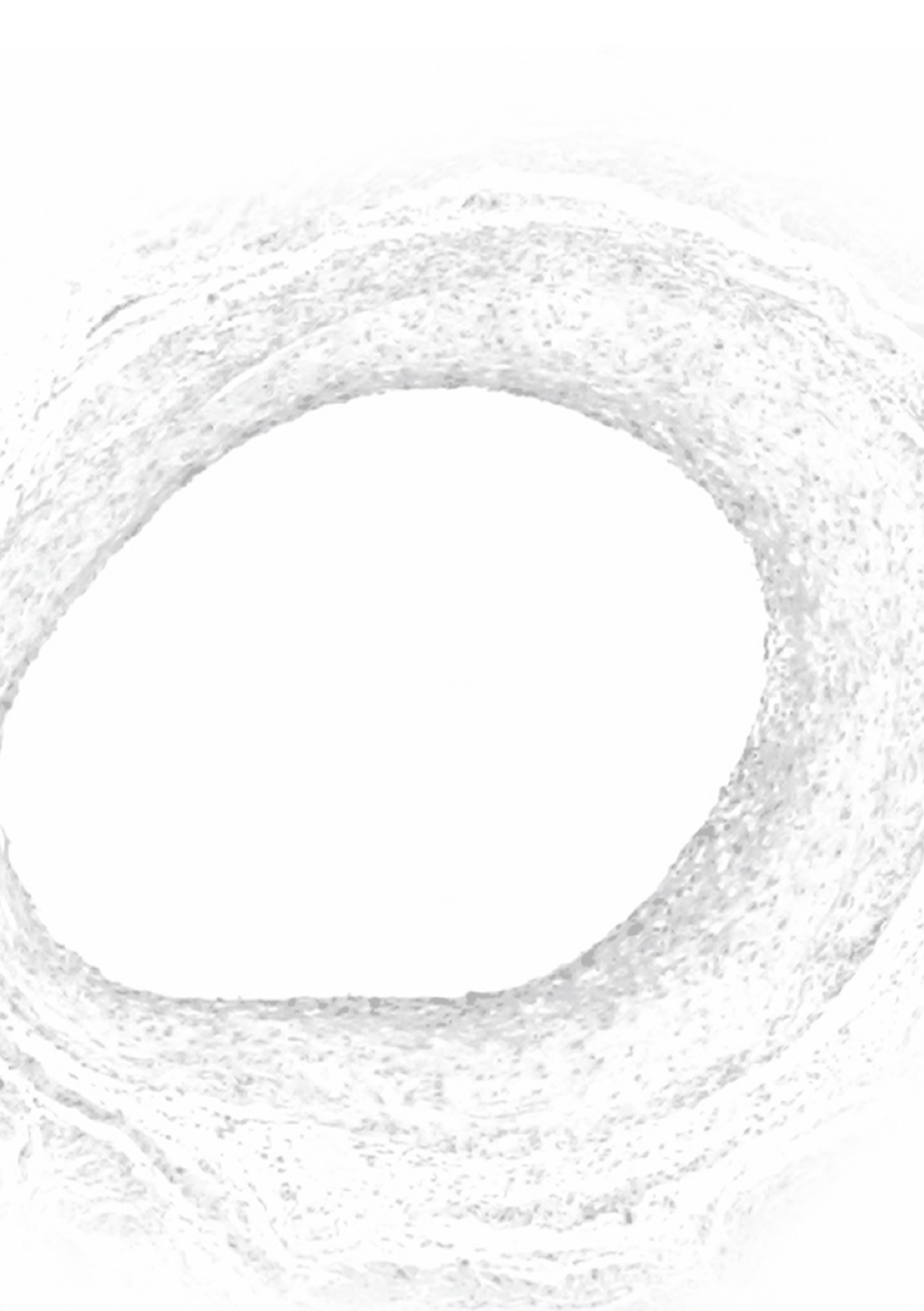
Inflammation in injury-induced vascular remodelling : functional involvement and therapeutical options

Schepers, A.



CHAPTER 2

Histopathologic Alterations Following Local Delivery of Dexamethasone to Inhibit Restenosis in Murine Arteries.

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ABSTRACT

Objective: Dexamethasone-eluting stents are currently under evaluation to prevent post-angioplasty restenosis. The efficacy and safety of dexamethasone as an anti-restenotic agent is still unclear. We assess the effect of perivascular delivery of dexamethasone on vascular pathology in a mouse model of restenosis.

Methods and results: In this study we investigate the ability of both systemic and local dexamethasone treatment to inhibit neointima formation after cuff placement around C57BL/6 mouse femoral artery. As in the clinical situation, systemic dexamethasone treatment shows adverse side effects in animals, including weight loss. In contrast, local delivery of dexamethasone using a drug-eluting polymer cuff inhibits neointima formation and has no systemic adverse effects. Pathobiological examination of the experimental arteries, however, reveals a dose-dependent medial atrophy, a reduction in vascular smooth muscle cells and collagen content, an increase in apoptotic cell count and disruption of the internal elastic lamina.

Conclusions: Our results demonstrate that although local dexamethasone delivery is effective as an inhibitor for neointima formation, it is dose-dependently associated with adverse vascular morphological changes pointing to a loss of vascular integrity.

INTRODUCTION

Percutaneous Transluminal Coronary Angioplasty (PTCA) has become the main treatment for revascularization of atherosclerotic coronary arteries in patients with (symptomatic) stenosis. The major drawback of this procedure, however, is the occurrence of restenosis[1]. Recently, drug-eluting stents were successfully introduced in interventional cardiology leading to an accentuated drop in the (in-stent) restenosis rate[2,3]. Many new anti-proliferative, anti-inflammatory, anti-migratory or pro-healing compounds are currently under evaluation to be loaded onto stents, including dexamethasone. Dexamethasone is widely used as a generic anti-inflammatory agent[4]. Dexamethasone is a glucocorticoid that exerts diverse inhibitory effects on several inflammatory-mediated responses and on smooth muscle cell (SMC) proliferation, two important events in the restenotic process[5-13].

In several animal models of restenosis, treatment with dexamethasone either locally[5,14-18] or systemically[6,19] demonstrated dissimilar outcomes on neointima formation inhibition depending on the animal model used and on the route of administration. Dexamethasone-eluting stents (DES) to prevent post-angioplasty restenosis have been developed recently and are currently under evaluation. Two short-term clinical trials using DES have been published showing conflicting results[20,21].

One well-defined mouse model of neointima formation consists of placement of a non-constrictive perivascular cuff around the mouse femoral artery, which results in a reproducible and concentric intimal thickening, mainly due to rapid induction of SMC proliferation[22-24]. Previously, we showed that the non-occlusive perivascular cuff to induce neointima formation could be constructed from a polymeric formulation suitable for controlled drug delivery[25]. This novel drug-eluting polymer cuff simultaneously induces reproducible intimal hyperplasia and allows locally confined delivery of anti-restenotic compounds to the cuffed vessel segment. This new approach gives the possibility to evaluate the effects of the tested compounds on neointima formation, vessel wall pathology and potential side effects[25].

In the present study we assessed the effect of systemic and local dexamethasone treatment on neointima formation inhibition in a mouse model of restenosis. More importantly, we evaluated the microscopic histopathologic alterations after local delivery of dexamethasone on arterial wall integrity and adverse vascular toxic effects.

METHODS

Femoral artery cuff mouse model.
For experiments, 12 weeks old male C57BL/6 mice were used. Animals were fed a standard chow diet (R/M-H, ssniff, Soest, Germany). Mice were bred and housed under specific pathogen-free conditions and given food and water ad libitum during the entire experiment. At the time of surgery, mice were anaesthetized with an

intraperitoneal (i.p.) injection of 5 mg/kg Dormicum (Roche, Basel, Switzerland), 0.5 mg/kg Dormitor (Orion, Helsinki, Finland) and 0.05 mg/kg Fentanyl (Janssen, Geel, Belgium). The femoral artery was dissected from its surroundings and loosely sheathed with a non-constrictive cuff [22-24].

All animal work was approved by TNO institutional regulatory authority and carried out in compliance with guidelines issued by the Dutch government. The investigation conforms with 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).

Systemic dexamethasone treatment.
Systemic dexamethasone treatment (0.30 mg/kg, LUMC Pharmacy, Leiden, The Netherlands) was achieved either by i.p. injections or oral therapy. Oral treatment was calculated for an average animal body weight of 30 g and a daily water consumption of 3 ml. Treatment was attained by adding dexamethasone to the daily drinking water throughout the whole experimental period, starting one day before surgery. The selected systemic dexamethasone dose was based on previous publications[6,19]. Eighteen mice were randomly divided into three groups according to the type of treatment (n=6/group). One group of mice was given daily i.p. dexamethasone injections, the second group was given dexamethasone in the drinking water, and the third group served as control and received normal drinking water and daily saline i.p. injections.

All mice underwent femoral artery cuff placement as described above and a polyethylene cuff (Portex, Kent, England, 0.40 mm inner diameter, 0.80 mm outer diameter, 2.0 mm length) was placed loosely around the murine femoral artery[22-24].

Dexamethasone-eluting PCL cuffs.
Powder dexamethasone was purchased from Sigma Diagnostics (St Louis, USA). Poly(ε-caprolactone) (PCL) based drug delivery cuffs were manufactured as described previously[25]. Dexamethasone-eluting PCL cuffs were made from blended molten drug-polymer mix and designed to fit around the femoral artery of mice. Drug-eluting PCL cuffs had the shape of a longitudinal cut cylinder with an internal diameter of 0.5 mm, an external diameter of 1 mm, a length of 2.0 mm and a weight of approximately 5.0 mg.

Mice underwent femoral artery cuff placement as earlier described. Either a control empty drug-eluting PCL cuff or a dexamethasone-eluting PCL cuff (1%, 5%, and 20% (w/w)) was used (n=6/group).

In vitro release profiles of dexamethasone.
PCL cuffs were loaded with 1%, 5%, and 20% (w/w) dexamethasone (n=5) and their in vitro release profiles were performed by UV-VIS (238 nm) absorbance methods as described previously[25,26]. Calibration graphs were established by measuring the absorbance of a set of standards in the 0-50 μg/ml concentration range.

Similarly, in vitro release profiles were also performed for dexamethasone-eluting stents (3.5 mm diameter, 22 mm length, Dexamet™, Abbott Vascular Devices Ltd, Ireland) as described above (n=2).

Quantification and histological assessment of intimal lesions in cuffed femoral arteries.
Animals were sacrificed 21 days after cuff placement. The thorax was opened and a mild pressure-perfusion (100 mmHg) with 4% formaldehyde in 0.9% NaCl (v/v) was performed for 5 minutes by cardiac puncture. After perfusion, femoral artery was harvested, fixed overnight in 4% formaldehyde, dehydrated and paraffin embedded. Serial cross-sections (200 μm; 5 μm thick) were used throughout the entire length of the cuffed femoral artery for histological analysis. All samples were routinely stained with hematoxylin-phloxine-saffron (HPS). Weigert´s elastin staining was used to visualize elastic laminae.

Ten equally spaced cross-sections were used in all mice to quantify intimal lesions. Using image analysis software (Leica Qwin, Wetzlar, Germany), total cross sectional medial area was measured between the external and internal elastic lamina; total cross sectional intimal area was measured between the endothelial cell monolayer and the internal elastic lamina.

Internal elastic lamina (IEL) disruption was assessed in all mice by evaluating the number of IEL rupture in ten equally spaced cross-sections throughout the entire length of the cuffed femoral artery segment.

Apoptotic cells were detected by terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick-end labeling (TUNEL) using in situ cell death detection kit (Roche Applied Science, Basel, Switzerland) according to the manufacturer instructions. Only TUNEL-positive nuclei that displayed morphological features of apoptosis including cell shrinkage, aggregation of chromatin into dense masses, and nuclear fragmentation were included. TUNEL-positive nuclei were counted in six equally spaced cross-sections in all mice and expressed as a percentage of the total number of nuclei.

Smooth muscle cells (SMC) were visualized with α-SMC actin staining (1:800, Roche Applied Science, Mannheim, Germany). Collagen content was determined using Sirius red stain. The amount of SMC and collagen was determined by morphometry as the total SMC-actin positive or Sirius red-positive area in six equally spaced serial cross-sections in all animals.

Statistical analysis.
All data are presented as mean±SEM. Data were analyzed using the Mann-Whitney U-test (SPSS 11.5 for Windows). *P*-values less than 0.05 were regarded as statistically significant.

RESULTS

Inhibition of neointima formation by systemic dexamethasone treatment.
We assessed the ability of systemic dexamethasone treatment, by either daily intraperitoneal (i.p.) boost injection or sustained oral therapy, to reduce cuff-induced neointima formation in our established mouse model of reactive stenosis. As shown in Figure 2.1A-C in control placebo-treated cuffed femoral, neointima was four to six cell layers thick, whereas in both systemic dexamethasone-treated groups cuff-induced neointima was maximally one or two cell layers thick. Morphometric analysis of the cuffed artery segments of both systemic dexamethasone-treated groups showed a comparable significant reduction on neointima formation (placebo treatment: 3.2±0.2; i.p. treatment: 1.3±0.2, *P*=0.002; oral treatment: 0.9±0.2x10^3 μm^2, *P*=0.002; Figure 2.1D). Moreover, intima/media ratios of the dexamethasone-treated groups were also significantly decreased (placebo treatment: 0.40±0.05; i.p. treatment: 0.16±0.02, *P*=0.002; oral treatment: 0.17±0.02, *P*=0.005) as compared to controls. This indicates that both systemic treatments similarly inhibited cuff-induced neointima formation.

With regard to side effects of systemic dexamethasone therapy, dexamethasone-treated animals showed adverse effects associated with dexamethasone systemic therapy, namely impaired wound healing and loss of muscular mass. Dexamethasone-treated animals lost weight during the 3 week-study period (i.p. treatment: pre-operative 29.0±1.0; sacrifice 25.4±1.5, P=0.04; oral treatment: pre-operative 26.2±0.7; sacrifice 21.6±1.4 g, P=0.02). Furthermore, clinical signs of delayed healing were present in 4 out of 12 dexamethasone-treated animals (2 in the i.p. treatment and 2 in the oral-treated group). No weight loss or impaired wound healing was observed in the control placebo-treated group. Moreover, general well-being of the dexamethasone-treated animals was also impaired, since treated animals with dexamethasone showed a profound decrease in daily activity and diminished fur quality. It should be noted that some of the above references (i.e. wound healing, fur quality and daily activity) are qualitative observation, since they are hardly quantifiable. Nevertheless, these observations are relevant indicators of possible side effects of dexamethasone systemic therapy.

Dexamethasone in vitro release profiles.
In order to overcome the adverse side effects observed with the systemic dexamethasone treatment we made use of a drug-eluting PCL cuff loaded with increasing concentrations of dexamethasone which allows restricted local perivascular delivery of compounds to the cuffed vessel segment.

PCL cuffs were loaded with 1%, 5%, and 20% (w/w) dexamethasone and their in vitro release profiles were determined for a 3-week period. Likewise, in vitro release profiles were also performed from dexamethasone-eluting stents for 21 days.

As shown in Figure 2.2, dexamethasone showed a sustained and dose-dependent release from the PCL cuffs for a 21-day period. Dexamethasone release was almost

complete for the lower loading dose (1%: 90.6±9.1%) whereas the cuffs with higher concentrations still contained dexamethasone (5%: 88.8±0.2%; 20%: 65.0±2.8%). Dexamethasone-eluting DEXAMETTM stents showed a comparable amount of drug released in the 21-day test period to the dexamethasone-eluting PCL cuffs (data not shown).

Figure 2.1: ***Representative cross-sections of cuffed murine femoral arteries.*** **A:** *Placebo treatment.* **B:** *i.p. dexamethasone treatment.* **C:** *Oral dexamethasone treatment. HPS staining, magnification 400x (arrow indicates the internal elastic lamina; arrowheads indicate the external elastic lamina).* **D:** *Total intimal area of cuffed murine femoral arteries 21 days after cuff placement. Total intimal area was quantified by image analysis using ten serial cross-sections from each cuffed artery and expressed in µm2 (mean±SEM, n=6). NS, P>0.05 (NS, not significant); **P<0.01.*

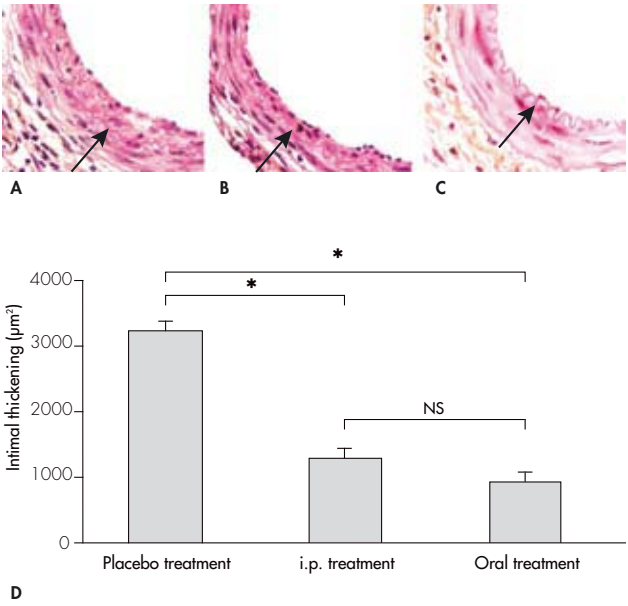
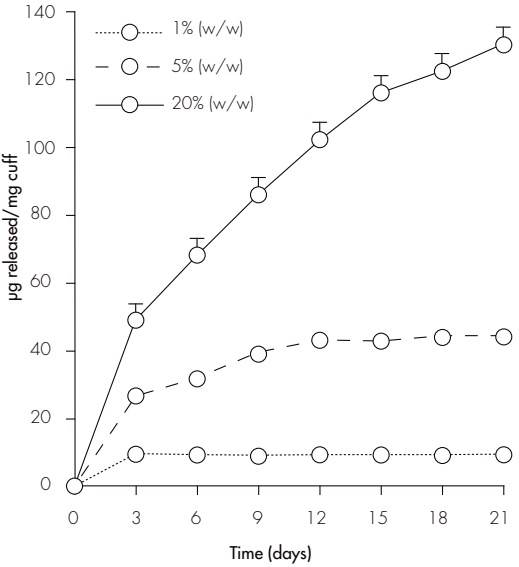
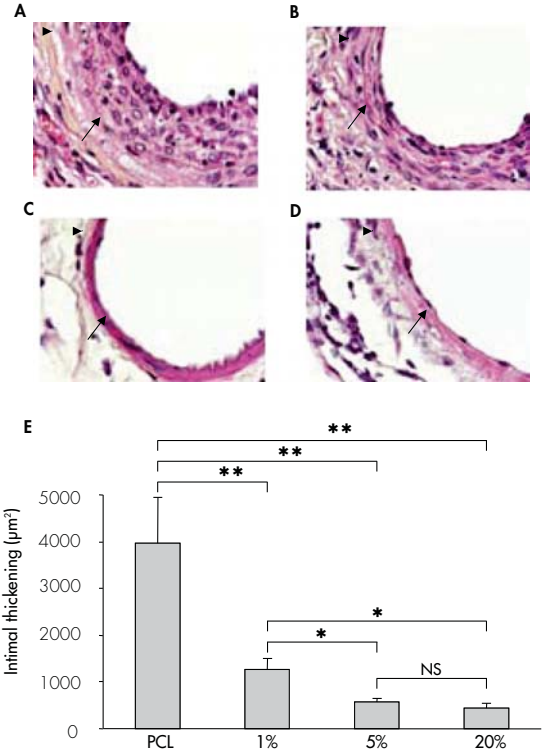


Figure 2.2: In vitro release profiles. *In vitro release profiles of dexamethasone-eluting PCL cuffs loaded with increasing concentrations of dexamethasone for a 21-day period (mean±SEM, n=5).*



Inhibition of neointima formation by perivascular dexamethasone delivery. To assess the effect of local perivascular dexamethasone delivery on cuff-induced neointima formation, PCL cuffs were loaded with 1%, 5%, and 20% (w/w) dexamethasone and placed around the femoral artery of male C57BL/6 mice for a 21-day period. Microscopic analysis of the cuffed femoral artery segments revealed that, after three weeks, a concentric neointima had been formed in mice receiving a control empty PCL cuff (Figure 2.3A). Most importantly, animals receiving a 1%, 5%, and 20% (w/w) dexamethasone-eluting PCL cuffs showed a strongly reduced intimal hyperplasia development or almost a complete absence of neointimal tissue (Figure 2.3B-D). Morphometric quantification by computer-assisted analysis revealed a significant and dose-dependent inhibition in cuff-induced neointima formation in all tested dexamethasone concentrations when compared with animals receiving a control empty PCL cuff (PCL cuff: 3.9±0.9; 1%: 1.2±0.2, *P*=0.007; 5%: 0.6±0.1, *P*=0.03; 20%: 0.5±0.1x10^3 µm^2, *P*=0.01; Figure 2.3E). Likewise, a similar dose-dependent decrease was seen in intima/media ratios of all dexamethasone loadings (PCL cuff: 0.35±0.12; 1%: 0.15±0.02, *P*=0.03; 5%: 0.13±0.01, *P*=0.03; 20%: 0.08±0.03, *P*=0.02) as compared to control empty PCL cuffed femoral arteries.

Figure 2.3: Representative cross-sections of cuffed murine femoral arteries treated with increasing concentrations of dexamethasone 21 days after cuff placement. **A:** *Control empty drug-eluting PCL cuff.* **B:** *1% (w/w) dexamethasone-eluting PCL cuff.* **C:** *5% (w/w) dexamethasone-eluting PCL cuff.* **D:** *20% (w/w) dexamethasone-eluting PCL cuff. HPS staining, magnification 400x (arrow indicates the internal elastic lamina; arrowheads indicate the external elastic lamina).* **E:** *Total intimal area of cuffed murine femoral arteries 21 days after drug-eluting PCL cuff placement. Total intimal area was quantified by image analysis using ten serial cross-sections from each cuffed artery and expressed in μm^2 (mean±SEM, n=6). NS, P>0.05 (NS, not significant); *P<0.05; **P<0.01.*



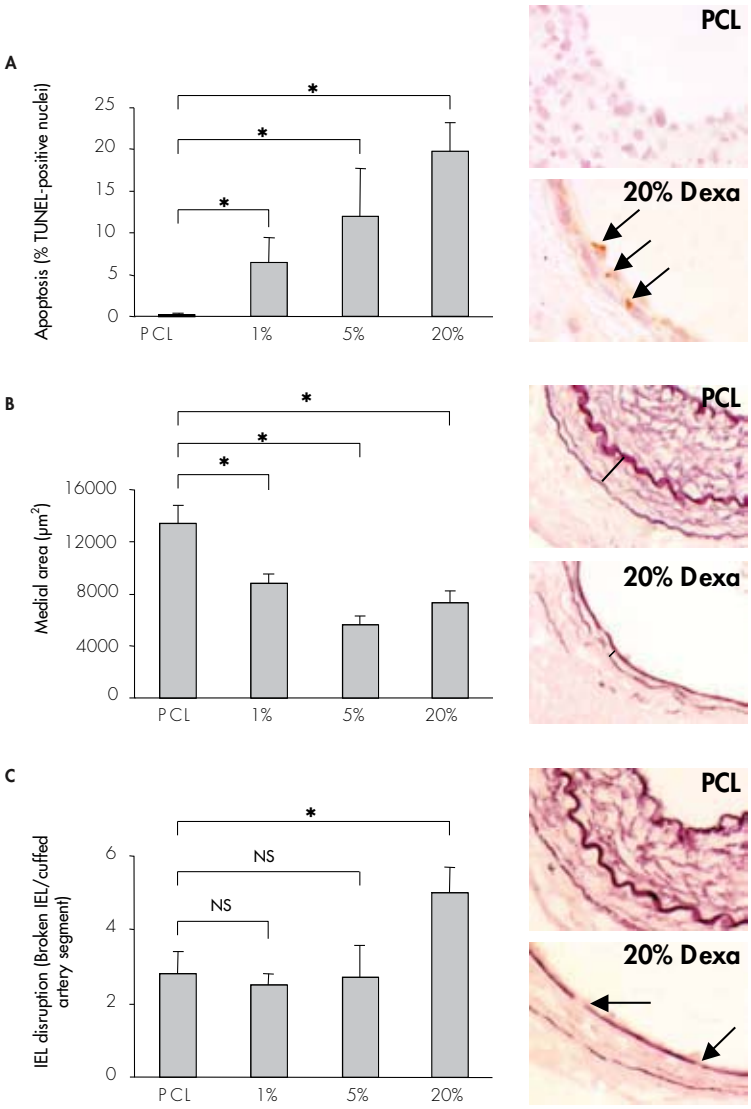
Animals receiving a 5% and 20% (w/w) dexamethasone-eluting PCL cuff showed local clinical signs of impaired wound healing in 1 and 2 out of 6 mice, respectively. No delayed healing was observed in the 1% (w/w) dexamethasone-eluting PCL cuff or in the control group. No significant changes in body weights were observed between any of the groups (data not shown). It should be noted that animals locally treated with dexamethasone did not show any impaired general well-being, contrary to what is observed after the systemic treatment.

Pathobiological evaluation of perivascular dexamethasone delivery on vessel wall integrity.
The overall structure of the media in the dexamethasone-treated mice had a changed appearance, pointing to an atrophic phenotype. This response demonstrated a dose-dependent reaction (Figure 2.3A-D).

To evaluate the adverse effects on the vessel wall of local perivascular delivery of increasing dexamethasone concentrations in more detail we first performed a standard TUNEL assay to assess apoptotic cells in the cuffed femoral artery segments after 21 days. As shown in Figure 2.4A, low levels of TUNEL-positive cells were seen in control empty PCL cuffed femoral arteries (0.3±0.1%). Nevertheless, increasing dexamethasone concentrations delivered locally to the vessel wall demonstrated a dose-dependent raise in the percentage of TUNEL-positive cells (1%: 7.3±3.2%, *P*=0.02; 5%: 12.0±5.7%, *P*=0.03; 20%: 19.7±3.5%, *P*=0.02). In the dexamethasone-treated arteries these TUNEL-positive cells were localized mainly in the media since there were hardly any cells forming a neointima. The increase in the number of apoptotic cells resulted in a significant medial cell loss in the dexamethasone-treated arteries (PCL cuff: 13.4±1.4; 1%: 8.8±0.7, *P*=0.03; 5%: 5.6±0.6, *P*=0.02; 20%: 7.3±0.9x10^3 µm^2, *P*=0.01; Figure 2.4B) as compared to control empty PCL cuffed arteries.

Figure 2.4: Histomorphometrical quantification of cuffed femoral arteries treated with increasing concentrations of dexamethasone. *Percentage of TUNEL-positive nuclei* **(A)***, total medial area* **(B)** *and internal elastic lamina (IEL) disruption* **(C)** *of cuffed femoral arteries treated with increasing concentrations of dexamethasone 21 days after drug-eluting PCL cuff placement. TUNEL-positive nuclei were counted in six equally spaced cross-sections from each cuffed artery and expressed as a percentage of the total number of nuclei. Medial area was quantified by image analysis using ten serial cross-sections in each cuffed artery and expressed in µm^2. IEL disruption was assessed in ten serial cross-sections from each cuffed femoral artery and expressed as the number of broken IEL per cuffed artery segment. Mean±SEM, n=6. NS, P>0.05 (NS, not significant); *P<0.05.*

Inserts: A: *TUNEL staining; femoral artery segments locally treated with 20% (w/w) dexamethasone (20% Dexa) show an increase in TUNEL-positive nuclei as compared to control empty PCL (PCL) cuffed segments. Arrows indicate TUNEL-positive nuclei, magnification 600x.* **B:** *Weigert's elastin staining; cuffed artery segments treated with 20% (w/w) dexamethasone show a striking medial atrophy. Bars indicate cross-sectional medial area, magnification 600x.* **C:** *Weigert's elastin staining; control empty PCL cuffed femoral arteries show an intact IEL while local delivery of dexamethasone enhances IEL disruption. Arrows indicate IEL disruption, magnification 600x.*

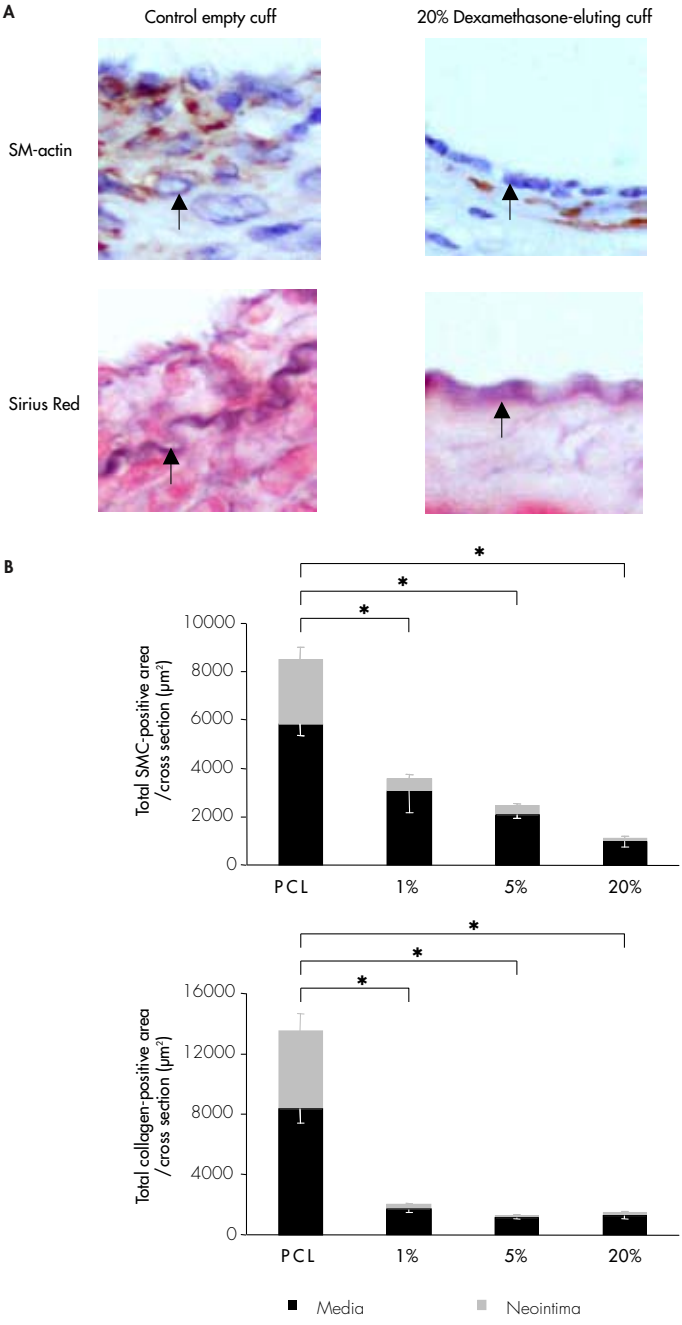

A
Apoptosis (% TUNEL-positive nuclei)
25
20
15
10
5
0
*
*
*
PCL
1%
5%
20%
PCL
20% Dexa
B
Medial area (μm²)
16000
12000
8000
4000
0
*
*
*
PCL
1%
5%
20%
PCL
20% Dexa
C
IEL disruption (Broken IEL/cuffed artery segment)
6
4
2
0
*
NS
NS
PCL
1%
5%
20%
PCL
20% Dexa

Vascular integrity was also assessed by evaluating the disruption of the internal elastic lamina (IEL) in the cuffed vessel segments. As depicted in Figure 2.4C, control empty PCL cuffed femoral arteries showed a number of IEL rupture similar to the one found in the lower concentrations of dexamethasone-eluting PCL cuffs (PCL cuff: 2.8±0.6; 1%: 2.5±0.3, *P*=0.2; 5%: 2.7±0.9, *P*=0.9). In contrast, the 20% (w/w) dexamethasone-eluting PCL cuff showed a 1.8-fold increase in IEL disruption (5.0±0.7, *P*=0.03) as compared to control empty PCL cuffed arteries.

Furthermore, total SMC content of experimental cuffed arteries was reduced in a dose-dependent manner when compared to control PCL cuffed vessels (PCL cuff: 8.5±0.5; 1%: 3.6±1.1, P=0.02; 5%: 2.5±0.2, P=0.03; 20%: 1.1±0.3x10^3 μm^2, P=0.01, Figure 2.5A and B). Similarly, dexamethasone-treated segments also showed a striking decrease in vascular collagen content when compared to control cuffed arteries (PCL cuff: 13.5±0.8; 1%: 2.0±0.3, *P*=0.02; 5%: 1.3±0.1, *P*=0.03; 20%: 1.5±0.2x10^3 μm^2, *P*=0.03, Figure 2.5A and B). Total SMC- and collagen-positive areas were assessed both in the media and in the neointima. As illustrated in Figure 2.5B, neointimal tissue showed a reduction in SMC and collagen content conceivably related to the intimal hyperplasia inhibition in the experimental arteries. Remarkably, medial tissue was also affected upon local dexamethasone treatment showing a striking decrease in both SMC and collagen content in the dexamethasone-treated arteries.

All dose-dependent vascular morphological changes observed in the local dexamethasone-treated arteries point to a loss of vessel wall integrity and adverse vascular toxic effects. Surprisingly, animals systemically treated with dexamethasone did not show any vascular adverse morphological changes like medial atrophy, increased apoptotic cell count or IEL fragmentation contrary to what is observed after local dexamethasone treatment (data not shown).

Figure 2.5: A: *Representative cross-sections of cuffed murine femoral artery 21 days after placement of either a control empty PCL cuff or a 20% (w/w) dexamethasone-eluting PCL cuff. Alpha smooth muscle cell actin staining for smooth muscle cells; a striking decrease in alpha SMC-positive cells content is observed in the cuffed vessel perivascularly treated with dexamethasone. Sirius red stain for collagen; a reduced vascular collagen content is present in vessel segments locally treated with dexamethasone. Magnification 600x (arrow indicates the internal elastic lamina).* **B:** *Total SMC- (top) and collagen-positive (bottom) area of cuffed murine femoral arteries treated with increasing concentrations of dexamethasone at 21 days after drug-eluting PCL cuff placement. SMC- and collagen-positive areas were quantified both in the media (black bars) and in the neointima (grey bars) by image analysis using six serial sections in each cuffed artery and expressed in μm^2. Mean±SEM, n=6. *P<0.05.*


A
Control empty cuff
20% Dexamethasone-eluting cuff
SM-actin
Sirius Red
B
Total SMC-positive area /cross section (μm²)
10000
8000
6000
4000
2000
0
PCL
1%
5%
20%
Total collagen-positive area /cross section (μm²)
16000
12000
8000
4000
0
PCL
1%
5%
20%
Media
Neointima

DISCUSSION

Drug-eluting stents coated with anti-restenotic drugs are a new promising tool to prevent post-angioplasty restenosis[27]. Several drugs have recently been coated onto stents to evaluate their potential as anti-restenotic agents. One of these drugs is dexamethasone, a glucocorticoid which is capable of inhibiting restenosis through several possible mechanisms. Firstly, glucocorticoids are potent anti-inflammatory agents that inhibit leukocyte adhesion and aggregation[7] and decrease the expression of several cytokines like monocytes chemoattratant protein-1 (MCP-1)[5,6,8], interleukin-1 (IL-1)[9] and interleukin-6 (IL-6)[5]. Secondly, they have a strong anti-proliferative effect and are able to inhibit SMC proliferation [10-13]. Finally, corticoids may decrease extracellular matrix deposition and remodeling through its ability to decrease collagen synthesis[28]. All these processes are believed to be important in the occurrence of restenosis.

Dexamethasone is a powerful agent that ideally should be administrated locally at the site of injury because of the detrimental side effects that are related to prolonged systemic administration. It is well known from clinical practice that prolonged systemic dexamethasone therapy frequently leads to altered body fat deposition, muscle atrophy, impaired wound healing and increased plasma lipid levels. Furthermore, similar side effects are described in mice exposed to dexamethasone treatment for a prolonged period of time (unpublished data, 2005).

In our study, systemic dexamethasone treatment during the experimental period inhibited cuff-induced neointima proliferation (60% inhibition with i.p. treatment and 71% inhibition with oral treatment) at the tested dose but was associated with systemic side effects including weight loss, reduced daily activity and decreased fur quality, all pointing to a significantly impaired general well-being (Figure 2.1).

In order to overcome these dexamethasone-related systemic side effects we made use of a drug-eluting poly(ε-caprolactone) (PCL) cuff to locally deliver dexamethasone[25]. Restricted local perivascular delivery of dexamethasone using a drug-eluting PCL cuff had no systemic side effects. The degree of neointimal growth inhibition was almost identical in the two higher concentrations (86% inhibition in the 5% and 89% in the 20% dexamethasone-eluting PCL cuff group) suggesting that a certain minimum amount of local drug concentration is necessary to inhibit intimal hyperplasia (Figure 2.3) and that higher concentrations might only raise local adverse side effects of the compound (Figure 2.4 and 2.5).

Several studies evaluating the local effect of dexamethasone on neointimal hyperplasia have been performed showing inconsistent outcomes. Periadvential dexamethasone delivery to the common carotid artery of rats significantly reduced intimal hyperplasia[14]. Conflictingly, the same drug delivery device failed to reduce neointima formation in a porcine model of restenosis[15]. Dexamethasone-eluting stents (DES) have failed to inhibit neointima formation in porcine coronary arteries[5,16] but proved to be successful in canine femoral arteries[17]. These divergent results might

be due to different dexamethasone concentrations or to species-related arterial differences.

Two published short-term human pilot studies using DES show conflicting results. The STRIDE trial (71 patients) using a BiodvYsio DES (dexamethasone dose of 0.5 μg/mm^2 of stent) reported a binary restenosis rate at 6-month follow-up of 13.3%[20]. Hoffmann and colleagues used a high-dose DES (2.2 μg/mm^2 of stent) for the treatment of de novo coronary lesions in 30 patients. At 6-month clinical follow-up the restenosis rate was fairly high with 31%, although this study included 17% of diabetic patients which are known to be predisposed to higher restenosis rates[21]. Independently of the intimal hyperplasia inhibition outcome none of the studies, either clinical or preclinical, focused on the potential vascular pathobiological effects of local delivery of dexamethasone.

It is suggested that the strongly hydrophobic character of the majority of the anti-restenotic drugs in use, such as dexamethasone, can lead to high arterial wall concentrations that exceed the bulk concentration[29]. This highly concentrated local delivery of potent anti-restenotic agents may lead to increased vessel wall toxicity. In our experiments, we indeed found a positive correlation between adverse morphological changes in the vasculature and the concentration of dexamethasone used. This indicates that increasing amounts of dexamethasone delivered to the arterial wall might not only inhibit neointima proliferation but also adversely affect the vessel wall exposed to the drug, leading to increased apoptosis and a loss of general vessel wall integrity.

Although the physical structure of our dexamethasone-eluting PCL cuff is somewhat different from that of a DES (the thickness of the polymer layer is different, the drug release is periadventitialy rather than intraluminarly and species-related arterial distinctions) attention should be given to vascular specimens treated with intracoronary DES, since we observe that the amount of dexamethasone released by a DES already invokes adverse toxic effects in murine arteries (Table 2.1). Our studies demonstrate that there is only a small therapeutic window in which a delicate balance between SMC proliferation inhibition and minimal vascular pathological side effects can be attained. This may have significant implications when initiating new clinical studies using higher doses of dexamethasone and at the clinical scenario of overlapping stents.

In conclusion, systemic and local dexamethasone therapy leads to an inhibition of neointimal growth in a murine model of vascular hyperplasia. Nonetheless, systemic dexamethasone treatment is associated with systemic side effects. Local delivery of dexamethasone leads to an abolition of the systemic side effects but was associated with medial atrophy, reduced vascular SMC and collagen content, increased number of apoptotic cells and internal elastic lamina fracture. These outcomes point to a loss of vascular integrity, particularly at high concentrations. The results of this study indicate that although local dexamethasone delivery has the potential to inhibit neointima formation, the toxic-therapeutic window in which the delicate balance

between maximal anti-proliferative effects and minimal effects on vascular pathology is relatively narrow since vascular pathobiological side effects are already detected at relatively low, but effective, doses of dexamethasone in the cuffs.

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