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Local Paclitaxel Delivery for the Prevention of Restenosis: Biological Effects and Efficacy In Vivo

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OBJECTIVE The aim of this study was to evaluate the potential of paclitaxel to prevent restenosis in vivo.

BACKGROUND Paclitaxel (Taxol) is a microtubule-stabilizing compound with potent antitumor activity. It influences the cytoskeleton equilibrium by increasing the assembly of altered microtubules, thereby inducing cellular modifications that result in reduced proliferation, migration and signal transduction.

METHODS Before the in vivo study, delivery efficiency was determined with radiolabeled paclitaxel in porcine hearts. After induction of a defined plaque in the right carotid arteries of 76 New Zealand rabbits by electrical stimulation, 27 animals underwent balloon dilation and subsequent local paclitaxel delivery (10 ml, 10 μmol/liter) with a double-balloon catheter. Twenty-nine animals served as control with angioplasty only, 10 animals underwent local delivery of vehicle only (0.9% NaCl solution) and 10 animals were solely electrostimulated. Vessels were excised one, four, and eight weeks after intervention.

RESULTS The extent of stenosis in paclitaxel-treated animals was significantly reduced compared with balloon-dilated control animals ($p = 0.0012$, one, four and eight weeks after intervention: 14.6%, 24.6% and 20.5%, vs. 24.9%, 33.8% and 43.1%, respectively). Marked vessel enlargement compared with balloon-dilated control animals could be observed ($p = 0.0001$, total vessel area after one, four and eight weeks: paclitaxel group: 1.983, 1.700 and 1.602 mm^2, control: 1.071, 1.338 and 1.206 mm^2, respectively). Tubulin staining and electron microscopy revealed changes in microtubule assembly, which were limited to the intimal area. Vasocontractile function after paclitaxel treatment showed major impairment.

CONCLUSIONS Local delivery of paclitaxel resulted in reduced neointimal stenosis and enlargement in vessel size. Both these effects contribute to a preservation of vessel shape and are likely to be caused by a structural alteration of the cytoskeleton. (J Am Coll Cardiol 2000;35:1969–76) © 2000 by the American College of Cardiology

The antitumor drug paclitaxel (Taxol) influences the cytoskeleton equilibrium by increasing the assembly of altered and extraordinarily stable microtubules and thereby reduces many cellular functions like proliferation, migration and signal transduction (1–3). It is highly lipophilic, which promotes a rapid cellular uptake, and has a long-lasting effect in the cell due to the structural alteration of the cytoskeleton (4,5). As shown previously, paclitaxel exerts potent and sustained inhibitory effects on smooth muscle cell (SMC) proliferation and migration in cell culture, even after single-dose application (6). This makes paclitaxel a very promising candidate for local drug therapy intended to address the proliferative and migratory processes involved in restenosis (6–8). The current study was designed to evaluate the effects of locally delivered paclitaxel in an experimental rabbit angioplasty model.

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METHODS

^{3}H-Paclitaxel delivery in porcine hearts. The efficiency of delivering paclitaxel with a double-balloon catheter was assessed ex vivo. In six freshly harvested porcine hearts, the left anterior descending artery (LAD) was canalized and a mid segment of the artery underwent balloon overstretch injury (12 atm, 20 s). Specific activity of the ^{3}H-labeled paclitaxel (Amersham International, Cardiff, England) was 6.65 Ci/mmol. A volume of 10 ml ^{3}H-paclitaxel (10 μmol/liter) was then delivered with the double balloon using an application pressure of 0.1 to 0.5 atm. The balloon-to-artery ratio was 1.2:1, mean application time was

Abbreviations and acronyms

ANOVA = analysis of variance
BD = balloon dilation
BRDU = bromodeoxyuridine
LAD = left anterior descending artery
LDD = local drug delivery
SMC = smooth muscle cell

7.2 ± 1.4 min. After rinsing the artery, the vessel segments and a block of surrounding myocardium were dissected. The specimens were individually homogenized over night in 1 ml Soluene (Packard, Groningen, the Netherlands) 350 + 0.3 ml H^2O^2 + 0.3 ml 2-propanol. After the addition of 15 ml Hionic-fluor (Packard), the resulting solution was transferred to a liquid scintillation counter for determination of the beta-emitting activity.

Local drug delivery device. A 2.2-mm double-balloon catheter (Schneider Europe, Bülach, Switzerland) was used for local drug delivery (LDD). It is an over-the-wire catheter with a 3-F shaft diameter and a distance between the two balloons of 20 mm.

Paclitaxel. Paclitaxel (Sigma, Deisenhofen, Germany) was dissolved in 100% ethanol and filter sterilized. The resulting stock solution (2.5 mM) was then diluted with sterile 0.9% NaCl solution to a concentration of 10 μmol/liter.

Animal study. The study protocol was reviewed by the ethical committee on animal research of our institution and was found to conform to the guidelines on animal care. Animals were housed in our facilities for two weeks before randomization to different treatment groups and inclusion in the study. During this period, one animal died of acute hepatitis, which was the only case of death in the whole study group. As detailed previously (9–11), the electrostimulation model was used to induce a fibromuscular plaque in the right carotid arteries of New Zealand rabbits. Briefly, this model is based on the implantation of two graphite-coated gold electrodes in the adventitia of the common carotid artery. Constant direct current impulses are then applied twice daily for a stimulation period of 28 days, and the animals are additionally fed a 0.5% cholesterol diet (Altromin, Lage, Germany). This combined procedure leads to an eccentric plaque growing below the anode. After the induction of eccentric plaque formation in 76 New Zealand rabbits, balloon dilation (BD) was performed in 66 animals under general anaesthesia (8 mg metomidate-HCL and 0.1 mg fentanyl base/kg body weight). A 2.0-mm A.C.S. (Santa Clara, California) Micro-Hartzler balloon was used for dilation (2 × 30 s, inflation pressure 6 atm). As control, 29 animals underwent BD only. LDD was performed in 27 animals with the double-balloon catheter and 10 ml of 10 μmol/liter paclitaxel. The double-balloon catheter was inflated with a pressure of 3 atm, and the drug was infused with a low pressure (0.1 to 0.5 atm) to allow for replacement of the escaping drug in the intervention area. The duration of LDD ranged from 8.6 to 13.1 min, with a mean of 9.8 min. As sham control group, 10 animals underwent LDD of vehicle only (0.9% NaCl solution) with the identical intervention parameters as in the paclitaxel group. Ten animals were only electrostimulated as a preinterventional control group. One, four and eight weeks after intervention, the animals were killed and the vessels were excised.

Bromodeoxyuridine (BRDU) labeling and histological preparation. In order to determine the extent of cells undergoing DNA synthesis, BRDU, a thymidine analogue, was given to each animal before the excision of the vessels, as described earlier (9). Before excision, the arteries were perfusion fixed in situ. After overnight immersion-fixation, the excised vessels were cut in five equal segments and embedded in paraffin. Each vessel segment was serially sectioned in seven slices of 4 μm each, at intervals of 75 μm. Each of the seven slices per interval was then stained with the different stainings as described in the following text. For quantitative analysis, at least three serial cuts at plaque maximum were used and then averaged per animal. These results were then finally averaged for the paclitaxel group and the three control groups.

Immunocytochemistry and histomorphometry. Immunohistological staining of cells with incorporated BRDU was performed by using a monoclonal antibody against BRDU (Bio Cell Consulting, Grellingen, Switzerland). Proliferating cells could be identified as smooth muscle cells (SMCs) with immunohistological alpha-actin staining (Renner, Dannstadt, Germany), and macrophages were detected with RAM 11 antibody (12,13). Immunofluorescent tubulin staining was performed with a monoclonal antibody against alpha-tubulin (Amersham Buchler, Braunschweig, Germany) that specifically stains polymerized tubulin (14,15). Controls were done by performing the identical staining procedure but omitting the first antibody. Cross sections were additionally stained with a polyclonal antibody against von Willebrand factor (goat anti-human factor VIII-related antigen; Atlantic Antibodies, Stillwater, Minnesota). In addition to the immunohistology, histological sections were stained with hematoxylin-eosin and elastica-van Gieson's stain. Each staining was performed in cross sections of each vessel segment. Two independent observers, blinded to the type of treatment protocol did the microscopical examination of the histological cross sections. Elastica-van Gieson's stained sections were quantitatively analyzed by computerized morphometry using standard software (Bioquant, Bilaney Consulting, Duesseldorf, Germany). The area of intima, media and residual lumen was calculated and the extent of stenosis was determined as follows: % stenosis = intimal area × 100/intimal + luminal area.

Transmission electron microscopy. Vessel segments for electron microscopy were fixed in 2% glutaraldehyde (Pae-

sel, Hanau, Germany) in 0.1 mol/liter cacodylate buffer, pH 7.4, for 1 h. The tissue was then washed several times in the same buffer, postfixed in 1% buffered osmium tetroxide for 1 h, washed again and dehydrated in ethanol. The specimens were then treated with saturated uranyl acetate in 70% ethanol for 2 h. After the completion of dehydration in absolute ethanol and propylene oxide, the tissue was embedded in Araldite (Serva, Heidelberg, Germany). Semithin and ultrathin sections were stained with toluidine blue and lead citrate, respectively. Ultrathin sections were examined with EM 10 and EM 902 microscopes (Carl Zeiss, Jena, Germany) and photographed.

Vascular contractile function. To investigate the effects of paclitaxel on vascular contractile function, rings of the left, untreated carotid artery of control animals were removed and prepared for further analysis. As described elsewhere in detail (16,17), the rings were then introduced into a contraction chamber for the measurement of vascular contraction force. Three different treatment groups were investigated (three vessel segments/group): 1) rings of carotid artery bathed in 10 μmol/liter paclitaxel for 10 min and investigated immediately; 2) rings bathed in 10 μmol/liter paclitaxel and then washed in tyrode for 2 h before measurement and 3) vessel segments incubated in 10 μmol/liter paclitaxel for 2 h. After prestretching the vessel rings with 50 mN and subsequent relaxation for 30 min, contraction was stimulated with isotonic salt solution in which KCl was increased to 30 mmol/liter, and contraction force was continuously registered. Using two identical measuring devices, every paclitaxel-treated vessel ring was simultaneously investigated with an untreated segment of the same artery as control.

Statistical evaluation. Results are expressed as mean ± standard deviation. In order to determine the significance of differences between paclitaxel-treated animals and balloon-dilated control animals, a two-way analysis of variance (ANOVA) was performed for each of the seven variables considering that the data were obtained at different time points. This statistical evaluation analyzes all the data for each variable at once and also reflects the interaction between therapy and the different time points when the results were obtained. Because of the additional control group, a separate one-way ANOVA was formulated for the results at day 7 after intervention. A complete list of the p values for the ANOVAs is presented in Table 1; most of the p values regarding the effect of therapy for the two-way ANOVA are presented in the text. The level of significance was set to 0.05. The statistical analysis for effects of therapy was adjusted for multiple testing according to Bonferroni-Holm. All tests were performed using SAS software (version 6.12; SAS Institute, Cary, North Carolina).

RESULTS

^{3}H-Paclitaxel delivery. After ^{3}H-paclitaxel delivery, 2.19 ± 0.77% of the initial dose of 10 ml paclitaxel solution

Table 1. Histomorphometry: Results of the Different Study Groups

	p Values					Study Groups					
Variables	One-way ANOVA at Day 7	Two-way ANOVA (Therapy)	Two-way ANOVA (Time)	Two-way ANOVA (Interaction)	Electro-stimulation Only (Pre-op)	Balloon Dilation (7 Days)	Sham Group: Vehicle (7 Days)	Local Paclitaxel Delivery (7 Days)	Balloon Dilation (28 Days)	Local Paclitaxel Delivery (28 Days)	Balloon Dilation (56 Days)	Local Paclitaxel Delivery (56 Days)
Luminal area (mm^2)	0.0913	0.0020	0.0376	0.2888	0.492 ± 0.274	0.654 ± 0.258	0.672 ± 0.189	1.153 ± 0.651	0.671 ± 0.277	0.787 ± 0.116	0.417 ± 0.226	0.754 ± 0.165
Intimal area (mm^2)	0.0155	0.1212	0.1928	0.8039	0.135 ± 0.088	0.210 ± 0.062	0.322 ± 0.117	0.179 ± 0.051	0.363 ± 0.306	0.261 ± 0.105	0.301 ± 0.188	0.193 ± 0.063
Medial area (mm^2)	0.0029	0.0001	0.1231	0.1304	0.404 ± 0.116	0.207 ± 0.081	0.280 ± 0.167	0.651 ± 0.295	0.306 ± 0.135	0.651 ± 0.165	0.466 ± 0.137	0.653 ± 0.155
Total vessel area (mm^2)	0.0148	0.0001	0.6450	0.1548	1.032 ± 0.325	1.071 ± 0.322	1.274 ± 0.307	1.983 ± 0.750	.338 ± 0.339	1.700 ± 0.177	1.206 ± 0.378	1.602 ± 0.295
Extent of stenosis (%)	0.0064	0.0012	0.0558	0.3091	22.5 ± 10.4	24.9 ± 10.0	32.7 ± 9.3	14.6 ± 6.9	33.8 ± 21.3	24.6 ± 7.3	43.1 ± 17.3	20.5 ± 5.3

Values are expressed as mean ± SD. ANOVA = analysis of variance. The statistical analysis for effects of therapy has been adjusted for multiple testing according to Bonferroni-Holm.

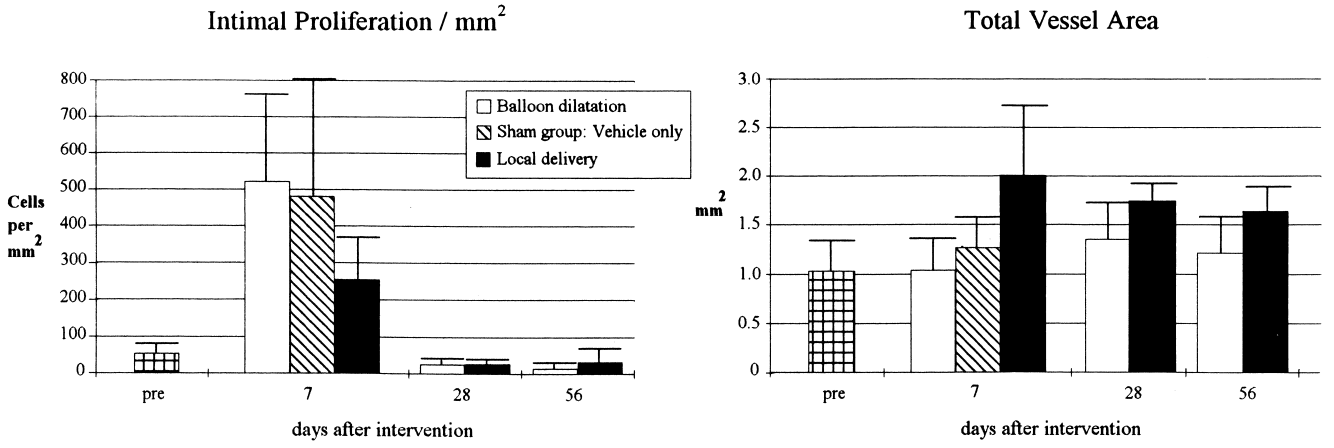

Figure 1. Extent of intimal proliferation and the total vessel area in the different groups. **Checked bars** represent the preinterventional control group, **white bars** stand for control animals that underwent BD only, **hatched bars** stand for sham animals that were treated with vehicle and **black bars** stand for paclitaxel-treated animals.

(10 μmol/liter) was detected in the vessel wall and 1.80 ± 1.21% was found in the adjacent myocardium. The relation of these results to the weight of the samples showed an activity of 43,286 ± 16,386 dpm/mg tissue in the LAD and 496 ± 287 dpm/mg tissue in the surrounding myocardium, equaling 24.59 ± 9.31 ng paclitaxel/mg tissue for the vessel wall as opposed to only 0.28 ± 0.16 ng/mg tissue in the adjacent myocardium.

Intimal proliferation and endothelialization. In control animals that underwent BD only, intimal proliferation at day 7 was increased to 522 ± 242 cells/mm^2 and in animals after local vehicle delivery to 483 ± 325 cells/mm^2. In contrast, intimal SMC proliferation was markedly reduced in paclitaxel-treated animals at day 7 (256 ± 116 cells/mm^2) compared with solely balloon-dilated animals. Four and eight weeks after intervention, intimal proliferation in all groups was similar to baseline levels (p value for effects of therapy: 0.0082, p value for effects of time: 0.0001, interaction: 0.0006). The exact data for all the different groups are given in Figure 1. Immunostaining for endothelial cells revealed no significant changes in endothelialization between paclitaxel-treated animals and control animals one, four and eight weeks after intervention (therapy: $p = 0.0400$, time: $p = 0.2680$, interaction: $p = 0.3125$).

Neointima formation and vessel size. The mean cross-sectional intimal area in vessels from paclitaxel-treated animals was constantly reduced compared with control animals; however, this reduction was not significant. After local paclitaxel delivery, the mean cross-sectional luminal area was significantly enlarged, with a p value of 0.0020 (seven days after LDD: 1.153 ± 0.651 mm^2, preinterventional baseline value: 0.492 ± 0.274 mm^2, balloon-dilated animals: 0.654 ± 0.258 mm^2, animals after local vehicle delivery: 0.672 ± 0.189 mm^2). Four and eight weeks after LDD, the differences in mean luminal area were still present (paclitaxel-treated animals: 0.787 ± 0.116 mm^2 and 0.754 ± 0.165 mm^2, solely balloon-dilated animals: 0.671 ± 0.277 mm^2 and 0.417 ± 0.226 mm^2). Corresponding to these results, the extent of stenosis in paclitaxel-treated animals was significantly reduced compared with balloon-dilated control animals ($p = 0.0012$, one, four and eight weeks after intervention: 14.6 ± 6.9%, 24.6 ± 7.3% and 20.5 ± 5.3%, vs. 24.9 ± 10.0%, 33.8 ± 21.3% and 43.1 ± 17.3%). A significant increase in the medial area was observed in treated animals compared with balloon-dilated controls ($p = 0.0001$). The total vessel area, measured as the area within the external elastic layer, was significantly larger in paclitaxel-treated animals compared with balloon-dilated control animals ($p = 0.0001$) (Figs. 1 and 2). No significant interaction between therapy and the different time points of observation was found for the histomorphological results. A detailed survey of all the results in the different study groups is listed in Table 1.

Morphological results. Local paclitaxel delivery with the double-balloon catheter resulted in only minimal additional injury. No specific alterations such as local intimal damages, disruptions in the internal elastic lamina, local medial thickening or medial fracture could be detected. Serial cutting revealed minimal intimal proliferation in areas where the two balloons of the double-balloon catheter were directly attached to the wall. This occurred in only 8 of 37 animals but could be clearly identified as a catheter-induced vessel wall alteration. Local medial compression and thinning with increased content of macrophages was detected in five animals seven days after intervention with the double balloon (three sham animals, two paclitaxel-treated animals). After local delivery of vehicle only, an increased occurrence of thrombus formation was found, with four mural or occlusive thrombi in 10 animals.

Tubulin staining showed an increase in the intensity of

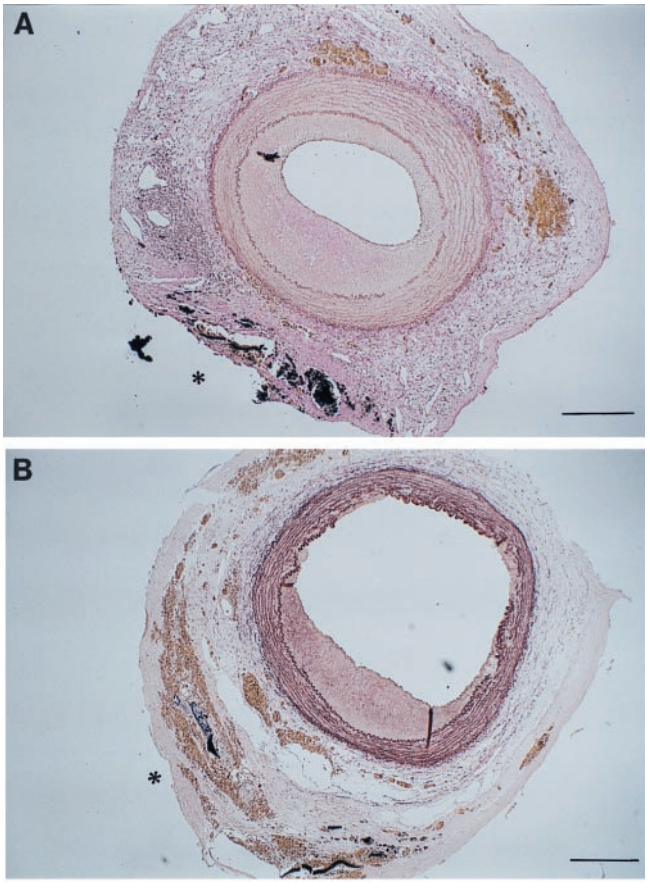

Figure 2. Photomicrographs of cross sections showing the effect of local paclitaxel delivery on vessel shape eight weeks after intervention. **A,** Control animal after BD showing a characteristic eccentric plaque formation with marked intimal thickening and lumen reduction. **B,** Histological section 56 days after local paclitaxel delivery showing the effects of paclitaxel on proliferation and vessel enlargement. *Remains of the graphite-coated gold electrode that had been loosely attached to the adventitia during electrostimulation. Elastica van Gieson staining. Scale bars represent 300 μm.

tubulin immunofluorescence, which was limited to the luminal cell lining and subintimal area. The increase in fluorescence intensity occurred in every cross-section of paclitaxel-treated animals seven days after intervention. However, this "fluorescence band" only showed in the area of the vessel exposed to paclitaxel during intervention. Even 28 days after intervention, intensive fluorescence in the luminal cell lining showed in about 40% to 50% of the histologic sections from paclitaxel-treated animals. This characteristic finding was not detected in any of the specimens from the control animals at any time (compare with Fig. 3). Specimens at the site of intense fluorescence were then prepared for electron microscopy to further characterize paclitaxel-induced vessel wall alterations. Electron microscopy showed microtubules both in control animals and in paclitaxel-treated animals. At very high magnification (125,000 ×), parallel microtubule bundles were detected in vascular SMCs that had been exposed to local paclitaxel delivery (Fig. 4). In contrast, no microtubule bundles were found in control animals.

Contractile function. After induction of vascular contraction with KCl-enriched solution, the contraction forces of both segments were measured. The contraction responses of paclitaxel-treated vessel rings were quantified compared with those of the respective controls. Incubation in 10 μmol/liter paclitaxel for 10 min led to a decrease in relative contraction force to 22.8 ± 1.4% compared with the control segments. This effect was preserved after washing the vessel segment in tyrode for 2 h (relative contraction force: 23.5 ± 2.1%). After the incubation of vessel segments in 10 μmol/liter paclitaxel for 2 h and immediate measurement, a further decrease in the relative contraction force (14.6 ± 4.5%) was observed.

DISCUSSION

The results of this study indicate that local paclitaxel delivery after experimental angioplasty leads to prolonged enlargement in vessel size and a reduction in neointimal stenosis. In addition to determining the amount of substance in the vessel wall, we were able to detect and document some of the biological effects of paclitaxel on the vasculature because of its unique mode of action.

Paclitaxel for LDD. Numerous pharmacological agents with antiproliferative properties have been tested for the potential to inhibit restenosis. Although initially promising, clinical results have mostly been disappointing (18). Even potent antimitotic compounds, such as methotrexate and colchicine, fails to inhibit SMC proliferation and intimal thickening in vivo (19,20). Although both paclitaxel and compounds of the colchicine type inhibit cell division in the M phase, opposite biological mechanisms are involved. In contrast to antimicrotubule agents like colchicine, which inhibit microtubule assembly, paclitaxel shifts the microtubule equilibrium toward assembly, thereby interfering with many functions of the cell such as proliferation, motility and migration, intracellular transport and transmembrane signaling (1–3,21). Moreover, activation processes associated with microtubule depolymerization (such as activation of protein kinases or release of transcription factors) are inhibited by paclitaxel, which seems to be of important functional relevance (22–24).

Paclitaxel has been shown to have an antiproliferative effect on rat SMCs in vitro, as well as in vivo (7). In a previous study, we could show that even after a short single-dose application (10 min) on human SMC cell culture, 10 μmol/liter paclitaxel exerted a sustained antiproliferative effect over a period of 14 days without showing rebound or cytotoxic effects (6).

Toxicity of paclitaxel. A number of toxic effects are known for paclitaxel in high-dose cancer therapy. The principal side effects are hematological toxicity (neutropenia), neurotoxicity (peripheral neuropathy) and hypersensitivity reac-

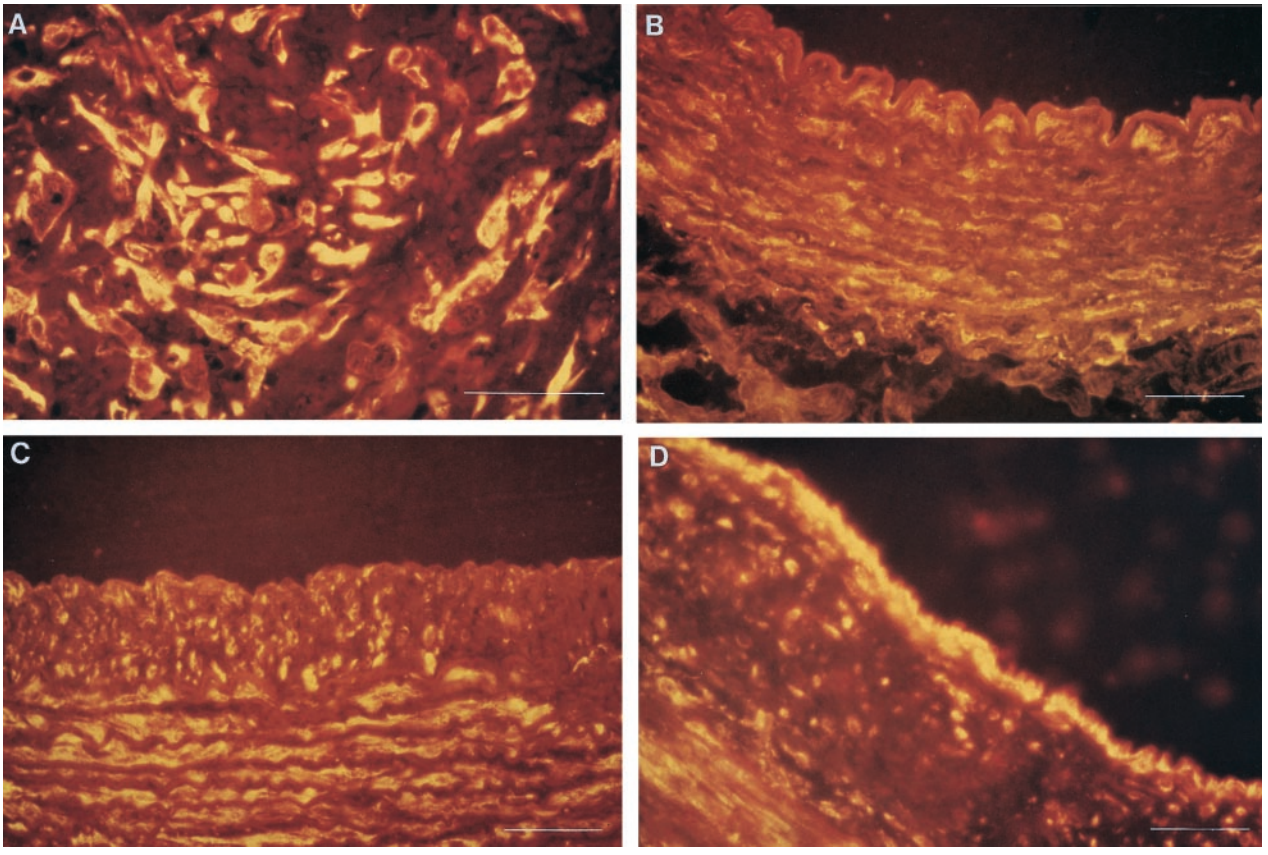

Figure 3. Immunofluorescence micrographs after staining with a monoclonal anti-tubulin antibody. **A,** Adventitia of a control animal seven days after angioplasty showing the specific staining of the cytoskeleton around the nucleus. **B,** Untreated control vessel after electrostimulation only. **C,** Control animal seven days after BD showing heterogeneous staining within the neointima. **D,** Histologic section seven days after local paclitaxel delivery showing an intensely stained "fluorescence band" at the luminal cell lining. Scale bars represent 50 μm.

tions. Cardiac disturbances (mostly transient asymptomatic bradycardia) have also been described, but all these side effects occurred in patients undergoing high-dose chemotherapy for a malignant disease (3,25,26). The plasma levels of paclitaxel in these patients are 100- to 1,000-fold higher (over a longer time period) than plasma levels that would result from a local infusion of 10 ml 10 μmol/liter paclitaxel. With a lower limit of measurement of about 50 nmol/liter, local paclitaxel delivery would not even result in measurable plasma levels in humans (27).

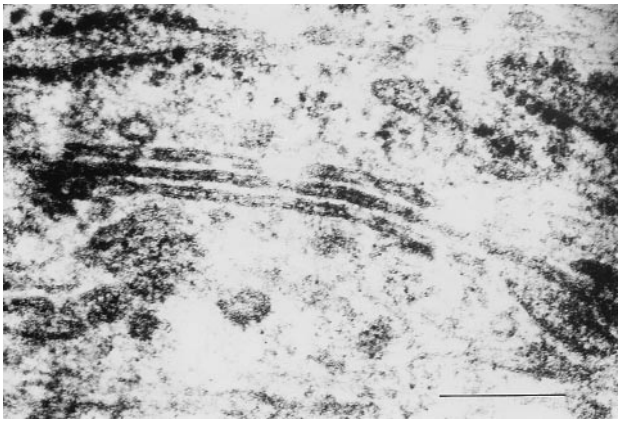

Figure 4. Transmission electron microscopy of an intimal SMC seven days after LDD showing a paclitaxel-induced microtubule bundle. Microtubule bundles were not found in control animals. Scale bar represents 0.2 μm.

Biological effects on the vessel wall and efficacy. In previous studies with other locally delivered drugs, efforts were made to determine the delivery efficiency by detecting the drug (or marker substances) in the vessel wall (28–31). In this study, 2.19% of the initially applied dose was detected in the vessel wall after the delivery of 10 ml radiolabeled paclitaxel (10 μmol/liter) with the double balloon. A comparison of these results with other studies is problematic due to different experimental approaches. It is known, however, that the efficiency of LDD is generally very low (32,33). At least in our own experience, the delivery efficiency of the applied combination of lipophilic drug and passive delivery system was 10- to 20-fold higher, when compared with a previous study using a hydrophilic compound (local delivery of low molecular weight heparin with the porous balloon) (32).

The more important question, however, would be the biological effects of this limited efficiency of LDD. The specific action of paclitaxel on the cytoskeleton allows for a unique feature: the detection of the drug action by staining of tubulin and electron microscopy. In paclitaxel-treated animals, tubulin staining showed an increase in fluorescence intensity, indicating changes in microtubule assembly. This gave reason for further investigations of the effects of paclitaxel on the vessel wall by electron microscopy. In fact, parallel microtubule bundles could be detected in SMCs that had been exposed to local paclitaxel delivery. It has been shown previously that these parallelly orientated microtubule bundles are a typical paclitaxel-induced modification in microtubule assembly (34,35). In control animals, no changes in the cytoskeleton were found at any time point.

The biological significance of these structural changes was assessed quantitatively in the different intervention groups. The morphological observation of an altered cytoskeleton within neointimal SMCs agrees well with the reduced intimal proliferation at an early stage after intervention in paclitaxel-treated animals compared with balloon-dilated control animals. In this study, reendothelialization after intervention did not differ significantly between paclitaxel-treated animals and control animals. In a previous study, we showed that higher concentrations of paclitaxel are needed to inhibit endothelial cell growth and SMC growth similarly (6). Because an intact endothelium is regarded as an important regulator for proliferation control, it is of great importance that these cell culture findings are preserved in the in vivo situation (36). The reduced neointima formation and extent of stenosis can simply be explained by the reduced intimal SMC proliferation after paclitaxel delivery. However, stenosis is not only caused by neointimal proliferation, but also by the reduction of residual lumen (37). After local paclitaxel delivery, the luminal area, medial area and total vessel area were consistently larger than in control animals. The reason for the observed medial hypertrophy in paclitaxel-treated animals remains unclear; however, this effect has also been described for other compounds (38). Theoretically, the effects on lumen and total vessel area might also have been caused by an additional dilation effect of the applied fluid between the two balloons of the double-balloon catheter. Therefore, the outer diameter of the vessel was carefully measured in situ and the carotid artery was only slightly mobilized during operation. With an infusion pressure of 0.1 to 0.5 atm, there was no additional dilation caused by the double balloon. In order to substantiate this observation, additional control animals were treated with local delivery of vehicle only, and the results in this study group also favor a drug effect and not an additional mechanical effect of the delivery system (compare with Table 1). Furthermore, vessel enlargement was also observed after a cytoskeleton-affecting therapy with other compounds, and the vasocontraction experiments of this study clearly indicate an effect caused by paclitaxel. Thus, the effects of paclitaxel on proliferation and vessel enlargement contribute both to a preservation of vessel shape and are likely to be caused by a structural alteration of the cytoskeleton. Paclitaxel does not act by a receptor-mediated mechanism and the highly lipophilic character of the drug promotes its cellular uptake. Additionally, the cytoskeleton is a well-preserved structure in evolution. Bearing in mind the pitfalls of transferring animal data into the clinical setting, this might be an advantage of a cytoskeleton-targeting therapy. Thus, local paclitaxel therapy with the double-balloon catheter might be a promising approach for the reduction of restenosis after percutaneous transluminal coronary angioplasty.

Study limitations. Local drug delivery in coronary arteries for a period of 10 min would require a device that allows for a residual blood stream through a perfusion lumen. A modified double-balloon catheter with a perfusion lumen is being developed. Even with the closed-chamber design of the double balloon, most of the delivered agent reaches the bloodstream, especially in coronaries with their side branches, and might cause adverse systemic side effects. Therefore, larger series of animal experiments as well as studies investigating local and systemic effects of paclitaxel in coronary arteries are desirable.

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