PRECLINICAL STUDY

Effects of Oral Prednisone After Stenting in a Rabbit Model of Established Atherosclerosis

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Objectives
The aim of this study was to compare the effects of systemic prednisone in combination with a bare-metal stent (BMS) or a paclitaxel-eluting stent (Taxus, Boston Scientific Corp., Natick, Massachusetts) on neointimal inhibition and vessel healing in an atherosclerotic rabbit model.

Background
Inflammation plays a critical role in neointimal formation after coronary artery stenting. The efficacy of immunosuppressive doses of oral prednisone to inhibit in-stent neointimal proliferation was compared with BMS and with a commercially available paclitaxel-eluting stent (Taxus) in a rabbit model of established atherosclerosis.

Methods
Bilateral iliac artery injury in atherosclerotic New Zealand White rabbits fed an atherogenic diet was followed by stent implantation. Animals randomly received Taxus stents, BMS (Express, Boston Scientific Corp.) and placebo, or BMS and oral prednisone (2.1 mg/kg/day for the first 7 days, followed by 1.4 mg/kg/day for 14 days and 0.7 mg/kg/day for 21 days). Stented arterial segments were harvested at 42 days and processed for light microscopy, immunohistochemistry, and organoid culture.

Results
Compared with control subjects, prednisone-treated animals showed a 30% reduction in percent stenosis (p < 0.009), a 35% decrease in neointimal area (p < 0.003), and a 66% decrement in neointimal thickness (p < 0.001). Taxus stents also reduced all 3 parameters significantly (<34%, <39%, and <83%, respectively), but showed significantly more inflammatory cells and fibrin deposition and less endothelialization compared with the other 2 groups. Plaque burden was equal among groups, as shown by the identical stent and vessel area, and no remodeling was observed.

Conclusions
Systemic prednisone treatment and Taxus stents reduce neointimal formation compared with BMS. The extent of neointimal reduction is similar between prednisone- and Taxus stent-treated animals; however, Taxus stents resulted in a significantly greater delay in healing. Targeting of inflammatory pathways after percutaneous coronary intervention may be an efficacious way to prevent restenosis without the long-term risk of late thrombosis. (J Am Coll Cardiol 2007;50:176–85) © 2007 by the American College of Cardiology Foundation

The local elution of cytostatic or cytotoxic drugs at vessel wall sites with drug-eluting stent (DES) technology has demonstrated efficacy, but it is limited by long-term delay in healing and risks of late stent thrombosis, as well as hypersensitivity reactions at the stented site (1,2). Targeting cell proliferation in a nonspecific manner using either paclitaxel or sirolimus significantly impairs many of the cellular processes involved in arterial repair after injury.

Because inflammation is of cardinal importance among the pathologic mechanisms of atherosclerosis and restenosis, interventions focused on reducing inflammatory signaling might attenuate restenosis without significantly delaying arterial healing. Only recently, the use of the oral corticosteroid prednisone after bare-metal stent (BMS) deployment has yielded significant reductions in restenosis in clinical trials (3,4). In fact, steroids exert beneficial effects on platelet function, smooth muscle cell proliferation, and collagen synthesis, as well as inflammatory cell migration and activation, thus interfering with several steps in the cascade leading to neointima formation and subsequent lumen loss (5). It is possible that steroids might achieve these benefits without the same risks of delayed healing.
and late thrombosis seen with current-generation DES. We therefore investigated the antirestenotic and anti-inflammatory properties of steroids after BMS implantation versus the current generation Taxus stent in an atherosclerotic rabbit iliac model.

**Methods**

This protocol was approved by the Institutional Animal Care and Use Committee of the Armed Forces Institute of Pathology and conformed to the position of the American Heart Association on use of animals in research.

**Rabbit model of experimental atherosclerosis.** The experimental preparation of the atherosclerotic animal model is depicted in Figure 1. New Zealand White rabbits (3 to 4 kg) 3 to 4 months of age were fed an atherogenic diet (1% cholesterol and 6% peanut oil, F4366-CHL, Bio-Serv, Inc., Frenchtown, New Jersey) for 5 weeks to induce atherosclerosis. Iliac artery injury was induced 1 week following induction of a high-cholesterol diet using a Fogarty catheter (3-F) as described previously (6). Following balloon injury, the animals were maintained on an atherogenic diet for 4 weeks. Subsequently the diet was switched to a low-cholesterol diet (containing 0.025% cholesterol) until euthanasia.

**Blood analysis.** Rabbits were phlebotomized and blood collected for the determination of total plasma cholesterol (Fig. 1). Serum C-reactive protein (CRP) measurements were performed using a highly sensitive 2-site enzyme-linked immunoassay. Time points of detection were 24 h and 48 h after stenting, 4 weeks after stenting, and at sacrifice (42 days).

**Stent placement and tissue harvest.** Premounted Express (control “bare” 316-L stainless-steel stent 3 × 18 mm) or Taxus stents (3 × 18 mm) (Boston Scientific Corp., Natick, Massachusetts) were implanted under fluoroscopic guidance. Stents were deployed bilaterally by inflation to their nominal pressures for 30 s. Following stent deployment, angiography was performed to document stent placement and vessel patency.

All animals received aspirin 40 mg/day orally until euthanasia. In addition, heparin (150 IU/kg) was administered intra-arterially before catheterization procedures. Forty-two days after stenting, animals were anesthetized, and a before-euthanasia angiogram of the iliac arteries was completed, followed by euthanasia and perfusion-fixation. The stented arteries were embedded in methylmethacrylate with sections taken from the proximal, middle, and distal portions of the stent. All sections were stained with H&E, Movat Pentachrome, and Carstair’s stains. To assess cellular proliferation, animals received bromodeoxyuridine (BrdU) before euthanasia, as previously described (6). Sections were also stained with antibodies to fibrin (American Diagnostica, Inc., Stamford, Connecticut).

**Drug treatment.** The animals were randomly allocated to 1 of 3 treatment groups: the first group received Express metallic stents and served as the control group, the second

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**Figure 1**

**Experimental Study Layout**

Time points of serum cholesterol measurements are highlighted (1 = day 7, 2 = day 35, 3 = day 63, 4 = day 84, and 5 = day 105).
group received Taxus stents (Taxus-treated group), and the third group received Express metallic stents followed by oral administration of prednisone (prednisone-treated group).

The administration of either placebo (groups 1 and 2) or prednisone (group 3) was started the day of stenting, maintained for 42 days, and administered as pills 3 times a day. The prednisone therapeutic scheme was as follows: 2.1 mg/kg/day for the first 7 days, followed by 1.4 mg/kg/day for 14 days and 0.7 mg/kg/day for 21 days.

**Organic culture.** In selected animals from each treatment group (n = 4 stents), stented arteries were explanted 7 days following deployment and perfused with ice-cold Ringer’s lactate at physiologic pressure (100 mm Hg). The specimens were harvested and immersed in fresh Dulbecco’s modified Eagle’s medium (DMEM) containing 0.1% bovine serum albumin (BSA). Vessels were cut into stented and nonstented portions and put in serum-free media (DMEM) containing 0.1% bovine serum albumin (BSA). Vessels were cut into stented and nonstented portions and put in serum-free media (DMEM) containing 0.1% bovine serum albumin (BSA).

**Cell culture.** Rabbit aortic smooth muscle cells were obtained from the American Type Culture Collection (Manassas, Virginia) and maintained in rabbit aortic smooth muscle growth medium (Cell Applications, San Diego, California). Following serum starvation in low-serum media (LSM) (DMEM + 1% fetal calf serum), migration assays were performed in 24 well plates using Falcon HTS Fluoroblock inserts (8-μm pore size; Becton Dickinson Labware, Franklin Lakes, New Jersey). The upper well was loaded with 350 μl of the cell suspension in LSM + DMSO or LSM + prednisone (100 μM). The lower well contained 700 μl of either LSM + DMSO (control), LSM + 100 ng/ml platelet-derived growth factor (PDGF) (BB isoform; BioSource International, Camarillo, California) + DMSO, or LSM + PDGF + prednisone. The migrated cells were labeled by adding 1 μl Calcein-AM to the lower well (1 mg/ml in dry dimethylsulfoxide, Molecular Probes, Eugene, Oregon). Pictures (100×) were taken on an inverted fluorescence microscope, and migrated cell fluorescence was quantified using Image J (7).

For investigation of cellular proliferation, rabbit aortic smooth muscle cells were serum starved and media subsequently replaced with either LSM + DMSO, LSM + 100 ng/ml bFGF (Invitrogen Corp., Carlsbad, California), or LSM + 100 ng/ml bFGF + prednisone (100 μM). At 0, 24, and 48 h, pictures were taken and quantified as previously described (8).

The impact of prednisone (100 μM; Sigma Aldrich, St. Louis, Missouri) or paclitaxel (0.01 μM; Sigma Aldrich) on re-endothelialization was assessed with a scratch assay using human umbilical vein endothelial cells as previously described (9).

**Data analysis.** All arterial segments were examined with the observer blinded to the treatment group. Stents were evaluated for thrombus formation, inflammation, and cellular proliferation. Computerized planimetry was performed (IP Lab, Vienna, Virginia) on all stented sections as previously described (6). The neointimal cell proliferation index (percent proliferating cells) was defined as the ratio of BrdU-positive neointimal cells to total neointimal cell number.

Fibrin was identified on H&E and Carstair’s stained sections and semiquantified as previously described (6,10).

### Table 1

**Comparison of Morphometric Measurements Among Treatment Groups 42 Days After Stent Implantation**

<table>
<thead>
<tr>
<th>Measurements</th>
<th>BMS (12 Stents)</th>
<th>Prednisone (12 Stents)</th>
<th>Taxus Stent (9 Stents)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>EEL area (mm²)</td>
<td>7.78 ± 1.41</td>
<td>7.50 ± 0.98</td>
<td>7.13 ± 0.91</td>
<td>NS</td>
</tr>
<tr>
<td>Stent area (mm²)</td>
<td>6.54 ± 1.01</td>
<td>6.02 ± 0.73</td>
<td>6.00 ± 0.66</td>
<td>NS</td>
</tr>
<tr>
<td>Plaque area (mm²)</td>
<td>1.23 ± 0.74</td>
<td>1.49 ± 0.58</td>
<td>1.13 ± 0.48</td>
<td>NS</td>
</tr>
</tbody>
</table>

**Measurements**

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Prednisone vs. BMS</th>
<th>Taxus vs. BMS</th>
<th>Taxus vs. Prednisone</th>
</tr>
</thead>
<tbody>
<tr>
<td>EEL area (mm²)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Stent area (mm²)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Plaque area (mm²)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

**BMS = bare-metal stent; EEL = external elastic lamina; NS = not specified.**

### Table 2

**Comparison of Morphometric Measurements of the Neointima Among Treatment Groups 42 Days After Stent Implantation**

<table>
<thead>
<tr>
<th>Measurements</th>
<th>BMS (12 Stents)</th>
<th>Prednisone (12 Stents)</th>
<th>Taxus Stent (9 Stents)</th>
<th>% Reduction and p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neointimal area (mm²)</td>
<td>2.21 ± 0.69</td>
<td>1.43 ± 0.38</td>
<td>1.34 ± 0.28</td>
<td>-35% (0.003)</td>
</tr>
<tr>
<td>% stenosis</td>
<td>34 ± 10</td>
<td>24 ± 6</td>
<td>23 ± 4</td>
<td>-30% (0.009)</td>
</tr>
<tr>
<td>Intimal thickness (mm)</td>
<td>0.29 ± 0.16</td>
<td>0.10 ± 0.06</td>
<td>0.05 ± 0.05</td>
<td>-66% (0.001)</td>
</tr>
</tbody>
</table>

**Abbreviations as in Table 1.**
Antifibrin stain was quantified as a percentage of neointimal positive area ([total neointimal positive area/neointima area] · 100).

Cytokine quantification was performed by densitometry using Gel-Pro-Analyser software (Version 4.5 for Windows, Media Cybernetics). Values for each cytokine were derived by extrapolation to a standard curve and normalization to the density of a provided negative control. Corresponding values from individual cytokines were compared among groups within proximal, stented, and distal sections.

Statistical analysis. Numerical data are presented as mean ± SD. Continuous variables were first checked for normal distribution by the Shapiro-Wilk goodness-of-fit test and

<p>| Table 3 | Comparison of Histologic Findings Among Treatment Groups 42 Days After Stent Implantation |</p>
<table>
<thead>
<tr>
<th>Measurements</th>
<th>BMS (12 Stents)</th>
<th>Prednisone (12 Stents)</th>
<th>Taxus Stent (9 Stents)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrin score</td>
<td>1.2 (0.9–1.4)</td>
<td>0.8 (0.7–1.0)</td>
<td>1.7 (1.5–2.0)</td>
<td>NS‡</td>
</tr>
<tr>
<td>% Neointimal fibrin</td>
<td>0.4 ± 0.3</td>
<td>1.3 ± 1.2</td>
<td>5.0 ± 3.6</td>
<td>0.004‡</td>
</tr>
<tr>
<td>% Endothelialization</td>
<td>83 ± 9</td>
<td>84 ± 10</td>
<td>64 ± 15</td>
<td>0.005</td>
</tr>
</tbody>
</table>

*Ordinal data are presented as median and interquartile range and analyzed by the Wilcoxon rank-sum test. Abbreviations as in Table 1.
analysis of variance or by the Wilcoxon rank-sum test performed where suitable. Dunnett’s or Bonferroni post-hoc adjustment was used to determine significant differences, and a p value \( <0.05 \) (\( p < 0.016 \) after Bonferroni correction for multiple, i.e., triple comparisons) was considered statistically significant. Ordinal data were analyzed by the Wilcoxon rank-sum test.

**Results**

Twenty-two animals formed the histomorphometric study, and 6 animals were used for the organoid culture. One animal from the prednisone group died during implantation because of procedural complications. Another animal from the Express control group was euthanized because of unacceptably high weight loss, lethargy, and icterus. Two rabbits from the Taxus stent group and 1 control rabbit were found dead 1 day following stent implantation. In all 3 animals, postmortem revealed iliac arterial dissections, likely secondary to stent implantation. All other animals appeared healthy, without weight loss for the duration of the protocol. Seventeen animals survived until scheduled euthanasia (6 prednisone, 5 Taxus, and 6 control animals).

Analysis of blood cholesterol levels revealed similar baseline parameters among treatment groups, with a mean serum-cholesterol of 640 ± 406 mg/dl for control, 991 ± 436 mg/dl for prednisone and 574 ± 365 mg/dl for Taxus stent rabbits (\( p = \text{NS} \)) at the time point of stent implantation (day 63). No significant differences were observed at sacrifice (data not shown). Serum CRP was significantly

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**Figure 3** Fibrin Presence in the Iliac Arteries 42 Days After Stent Implantation and Bar Graph for Percentage of Fibrin Area

Photomicrographs showing fibrin deposition around stent struts using Carstair’s stain (A to C) (pink color), stain with antibody against fibrin (D to F) (brown color) and hematoxylin-eosin (G to I) in control animals (A, D, G), prednisone-treated animals (B, E, H) and Taxus stents (C, F, I). Note that maximum fibrin is seen in Taxus stents after 42 days. (J) Bar graph showing significantly greater percentage of fibrin area in Taxus stents compared with control or prednisone-treated animals.
reduced in prednisone-treated rabbits 24 h after stenting compared with Taxus stent-treated rabbits and was similar to control rabbits at this time point (73.37 ± 12.35 ng/ml vs. 106.81 ± 5.56 ng/ml, p < 0.04 and 80.46 ± 21.39 ng/ml [control], p = NS). The CRP levels remained significantly lower in prednisone-treated rabbits at 48 h; however, no significant differences were observed at 4 weeks and at the time point of sacrifice (day 105).

**Histomorphometry.** Histomorphometry was performed in 12 Express stents deployed in 6 placebo-treated rabbits (control group), 12 stents deployed in 6 prednisone-treated rabbits, and 9 Taxus stents deployed in 5 rabbits (1 stent was totally occluded and excluded from analysis).

The experimental baseline conditions created in the 3 groups were identical in terms of external elastic lamina area, stent area, and plaque area (Table 1). Arterial injury was mild in all treatment groups (mean injury score <1) (data not shown).

Oral immunosuppression with prednisone resulted in a significant reduction in neointimal area, thickness between struts, and percent stent stenosis compared with control animals (−35%, −66%, and −30% reduction, respectively). Taxus stents also showed highly significant differences compared with control animals in terms of these end points (−39%, −83%, and −34% reduction, respectively) (Table 2, Fig. 2). Taxus stent deployment resulted in statistically similar results compared with prednisone treatment when we examined reduction in neointimal area and percent stent stenosis, but was superior in terms of suppression of neointimal thickness (−50% reduction compared to prednisone, p = 0.02) (Table 2). There was almost complete endothelialization and neointimal coverage around stent struts in control and prednisone-treated animals, whereas Taxus stent-treated arteries showed incomplete healing, characterized by large amounts of fibrin deposition and uncovered stent struts.

### Table 4
**Comparison of Proliferation Index and Luminal Eosinophils/Heterophils Among Treatment Groups**

<table>
<thead>
<tr>
<th>Measurements</th>
<th>BMS (12 Stents)</th>
<th>Prednisone (12 Stents)</th>
<th>Taxus Stent (9 Stents)</th>
<th>Prednisone vs. BMS</th>
<th>Taxus vs. BMS</th>
<th>Taxus vs. Prednisone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean BrdU index</td>
<td>0.08 ± 0.05</td>
<td>0.10 ± 0.04</td>
<td>0.05 ± 0.03</td>
<td>NS</td>
<td>NS</td>
<td>0.005</td>
</tr>
<tr>
<td>Mean no. of eosinophils/heterophils</td>
<td>32 ± 24</td>
<td>27 ± 20</td>
<td>72 ± 33</td>
<td>NS</td>
<td>0.01</td>
<td>0.001</td>
</tr>
</tbody>
</table>

BrdU = bromodeoxyuridine; other abbreviations as in Table 1.

**Figure 4**
**Inflammation at Luminal Surfaces and Bar Graph for the Heterophils/Eosinophils**

Demonstration of luminal eosinophils/heterophils after staining with H&E at high (200×, insets at 400×) magnification. Note, greatest number of eosinophils/heterophils are seen in Taxus stents (C) versus prednisone-treated (B) and control (A) animals. (D) Bar graph demonstrating significant differences in luminal inflammatory cells among treatment groups.
The fibrin score was significantly higher in the Taxus stent group compared with control subjects (1.7 [interquartile range (IQR) 1.5 to 2.0] vs. 1.2 [IQR 0.9 to 1.4], p < 0.003) and this difference was even more pronounced when compared with prednisone treated animals (1.7 [IQR 1.5 to 2.0] vs. 0.8 [IQR 0.7 to 1.0], p < 0.008) (Table 3). Following immunostaining for fibrin, Taxus stents showed significantly greater fibrin deposition compared with prednisone-treated and control rabbits (Taxus vs. control 5.0 ± 3.6% vs. 0.4 ± 0.3%, p < 0.03; prednisone-treated vs. control 1.3 ± 1.2% vs. 0.4 ± 0.3%, p = NS; and Taxus vs. prednisone-treated 5.0 ± 3.6% vs. 1.3 ± 1.2%, p < 0.05) (Fig. 3).

Inflammatory cells consisted mainly of macrophages and lymphocytes with minor giant cell reaction, which were equally observed in all three groups. However, in Taxus stents, the mean number of eosinophils/heterophils toward the lumen was significantly greater compared with control and prednisone-treated animals (Taxus 72 ± 33 vs. control animals 32 ± 24, p < 0.01 and prednisone 27 ± 20, p < 0.001) (Table 4, Fig. 4).

Neointimal cell proliferation was lowest in Taxus stents and was significantly less compared with prednisone–treated rabbits (p < 0.005), whereas no significant difference was observed between prednisone–treated and control rabbits (Table 4).

Cytokine array of organoid cultures. Cultured stented arteries predominantly produced immunogenic and chemoattractant cytokines such as interleukins and growth factors at 7 days. Most of these released cytokines, detected by individual spots on X-ray films, showed similar response patterns among the 3 treatment groups, demonstrating reduced release in both the prednisone and Taxus as compared with control arteries. Significant decrease among prednisone or Taxus compared with control arteries was observed for interleukin-6, -10, and -13; monocyte chemoattractant protein-1, -2, and -3; regulated on activation, normal T cell expressed and secreted; transforming growth factor-beta; and tumor necrosis factor-alpha (Fig. 5). Cellular proliferation and migration in vitro. Rabbit aortic SMCs treated with prednisone demonstrated significantly diminished migration (1.67 ± 0.45-fold, p < 0.03) compared with untreated but stimulated (PDGF) cells (2.92 ± 0.38-fold). There was no difference in proliferation between cells treated with bFGF alone and bFGF and prednisone.

Re-endothelialization in vitro. Re-endothelialization following scratch injury was significantly delayed in paclitaxel-treated endothelial cells compared with control cells (p < 0.02). This delay was even more pronounced when compared with prednisone-treated endothelial cells (p < 0.003). Most important, there was no delay in re-endothelialization in prednisone–treated cells compared with control cells (p = NS) (Fig. 6).

Discussion
This study demonstrates that, in the experimental model of atherosclerotic rabbits, oral administration of prednisone causes a significant 30% reduction in percent stent stenosis and allows for complete re-endothelialization.
after implantation of BMS. In vitro findings with endothelial cells confirmed that prednisone does not interfere with re-endothelialization following scratch injury at relatively high concentrations. Although the Taxus stent caused greater neointimal inhibition, it also resulted in histologic evidence of incomplete healing characterized by persistent fibrin deposition, inflammation, and incomplete endothelialization in vivo and in vitro.

The concept that systemic administration of immunosuppressant agents such as prednisone may reduce neointimal formation after coronary stent placement without impeding arterial healing is an important one. Although current-generation DES are effective in reducing rates of target vessel revascularization, they do so at the cost of inhibition of arterial healing, which requires long-term dual antiplatelet therapy to reduce the risks of thrombosis. Multiple studies have confirmed that the most important risk factor for late stent thrombosis after DES placement is withdrawal of antiplatelet therapy. We recently reported (11) a long-term delay of arterial healing after DES implantation in humans, characterized by fibrin deposition, inflammation, and poor endothelialization as the pathologic substrate underlying late stent thrombosis.

The experimental animal model. Unlike prior experimental studies performed with the commercially available DES, our investigation was performed in atherosclerotic animals, which offer a more realistic setting for the assessment of

Figure 6 In Vitro Scratch Assay With Human Umbilical Vein Endothelial Cells and the Bar Graph of Cell Density

Representative photomicrographs of scratch injury following treatment with either low serum essential culture media (LSECM) + dimethyl sulphoxide (control) (A), LSECM + prednisone (B), or LSECM + paclitaxel (C) (0.01 μM). Graphic analysis of scratch assay indicates that there is no significant difference between endothelial cell density increase values for the control and prednisone treatment conditions (D). There is a significantly less increase in cell density in the paclitaxel treatment condition (53.68 cells/area ± 12.29) compared with both the control (209.89 ± 32.06) and the prednisone (260.16 ± 51.40) treatment conditions.
percutaneous interventions in preclinical studies (1,2). In the specific setting of DES implantation, there are concerns that the extraordinary efficacy observed in the short term in healthy animals (and supported by initial clinical experience in humans) may not be sustained in the long term (12). In our study, prednisone-treated animals received tapered doses of steroids to reproduce human protocols and to minimize possible undesirable effects, and the histomorphometric analyses were obtained at midterm (42 days) rather than at 28 days.

Moreover, the development of an atherosclerotic plaque before stenting ensures a more aggressive neointimal response with an important inflammatory reaction compared with healthy animal models. The efficacy of prednisone observed in our experiment is most likely related to its anti-inflammatory properties, whereas Taxus stents coated with an antiproliferative drug have undesirable effects resulting in persistent fibrin deposition, poor endothelialization, and induction of an eosinophilic/heterophilic inflammatory response.

Antirestenotic mechanisms of glucocorticoids. Glucocorticoids are well known to suppress several inflammatory pathways by interacting with NF-κappa B, thus blocking its transcriptional activity (13,14). The reduced transcription of various proinflammatory genes results in diminished release of inflammatory cytokines, chemokines, and cell adhesion molecules. Our study confirmed a markedly reduced release of inflammatory cytokines in stented arteries 7 days following prednisone treatment, which likely contributed to the beneficial effect on neointimal growth observed at midterm follow-up (42 days). In contrast, paclitaxel is known to interrupt mitosis by promoting and stabilizing microtubule formation and, therefore, acts as an antiproliferative drug, with minor effects on arterial inflammation. The diminished release of inflammatory cytokines obtained at 7 days with Taxus stents in our study might therefore be explained by toxic effects on vascular cells, resulting in decreased cellular viability rather than suppression of the inflammatory reaction. The histologic response at 42 days, however, suggests a catch-up phenomenon of neointimal formation, as demonstrated by sustained fibrin deposition and eosinophilic/heterophilic infiltration.

The antiproliferative effects of glucocorticoids are well characterized, and there is consensus that persisting high levels are necessary to efficiently inhibit proliferation of vascular smooth muscle cells (15). In the current study, in vitro experiments revealed an absence of antiproliferative effects at concentrations comparable to those used in vivo. However, there was a clear effect on migration of SMCs, which might occur independent of antiproliferative effects at relevant concentrations. Most important, re-endothelialization was not delayed in vitro using the same concentrations of prednisone that were shown effective in suppressing migration of SMCs. Further investigation is needed to determine the differential response of vascular SMC and endothelial cells to prednisone treatment.

**Comparison with other preclinical studies.** A previous animal study (16) reported a significant decrease of inflammation following implantation of dexamethasone-eluting stents in pig coronary arteries at 5 days. However, no beneficial effect on neointimal growth was observed at 28 days, which reinforces the need for appropriate animal models and drug dosage to simulate human atherosclerotic disease.

Furthermore, the local or systemic administration is of cardinal importance to determine the efficacy of glucocorticoids with respect to their antirestenotic properties. In contrast to corticosteroids, preclinical models using paclitaxel have shown effective suppression of neointima at short term. In a previous experimental model of healthy rabbits implanted with stents loaded with a lower dose of paclitaxel (42.0 μg), neointimal thickness was reduced by 48% compared to BMS at 28 days (12). However, as in our study, paclitaxel-DES were associated with significantly larger areas of fibrin deposition surrounding stent struts and sparse coverage of neointima with signs of incomplete healing. These morphologic findings suggest local toxic effects of paclitaxel, which were no longer apparent at 90 days, and neointimal thickness was similar to control BMS (12). In a recently published investigation, high-dose paclitaxel-loaded stents (1.44 μg/mm²) implanted in healthy pigs confirmed a 50% reduction of neointima compared with control subjects at 28 days, but also a total loss of the inhibitory effect on the neointima at 90 days (17).

In the present experiment, the commercially available Taxus stent, loaded with 1.0 μg/mm² of paclitaxel, reduced percentage of stent stenosis very effectively (−34%) at 42 days in the setting of an underlying atherosclerotic plaque burden, confirming the efficacy of the Taxus stent at the midterm. As in previously mentioned studies, the Taxus stent was associated with significantly larger areas of fibrin deposition (Fig. 3), inflammatory cells surrounding stent struts, and sparse coverage of neointima by endothelial cells, resulting in incomplete healing.

**Comparison with clinical studies.** Quantitative coronary analysis of patients with implanted Taxus stents in clinical trials have demonstrated a late loss to vary between 0.36 ± 0.48 mm and 0.39 ± 0.50 mm at 6 to 9 months compared with approximately 0.92 ± 0.58 mm in BMS control patients (18,19). In-stent late loss observed in BMS and oral prednisone in the IMPRESS studies varied from 0.39 ± 0.06 mm to 0.61 ± 0.35 mm at 6 to 8 months compared with 0.85 ± 0.6 mm in control patients (3,4). These angiographic results seem to encompass the morphologic observations described in our animal study and may be sufficient to avoid clinical recurrence. In fact, it is now accepted that in-stent late-loss values <0.75 mm practically avoid the hazard of clinical restenosis and that further reductions of late loss may be clinically meaningless (20).

The drawback to this approach is its limited applicability. Only 15% of patients in the former study fulfilled enrollment criteria. In the real world, the well-known unfavorable side effects of oral prednisone, such as hypertension, im-
paired glucose tolerance, immunosuppression, impaired healing after myocardial infarction, and increased risk for gastric ulcers would preclude its use in some patients coming to the catheterization laboratory because of the high incidence of comorbid conditions, such as diabetes or congestive heart failure, that would only be exacerbated by steroid therapy. On the other hand, there are also important benefits to oral prednisone not provided by other approaches, such as DES, and these include low cost of treatment and lack of long-term need for dual antiplatelet therapy after percutaneous coronary intervention. These advantages, especially the latter, should not be understated, especially because the optimal duration of antiplatelet therapy after DES is yet to be determined.

**Study limitations.** Our study does not address possible differences between mid- and long-term results of the efficacy of oral prednisone. However, on the basis of the relatively favorable histologic findings observed with oral prednisone (complete healing, absence of fibrin deposits, and less inflammation compared with the paclitaxel DES), it seems unlikely that the long-term result of this strategy could be worse than those reported here. However, further research is warranted to address this issue.

**Conclusions**

Oral administration of immunosuppressive dose of prednisone for 42 days following BMS implantation in atherosclerotic animals, as used in clinical studies, results in a significant reduction in percent stenosis (i.e., neointimal growth) compared with BMS. The magnitude of the antirestenotic effect of prednisone observed in this experimental model is similar to that obtained with Taxus stents, but with a more favorable healing aspect. These findings support the encouraging clinical and angiographic findings reported in preliminary clinical studies. However, in making clinical decisions about whether to use adjunctive steroid therapy after percutaneous coronary intervention, one must weigh the well-known adverse side effects of oral prednisone therapy against its potential benefits as an antirestenotic agent that does not appear to delay healing (at least in preclinical models).

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