Pharmacokinetic Study of Paclitaxel Concentration after Drug-Eluting Balloon Angioplasty in the Iliac Artery of Healthy and Atherosclerotic Rabbit Models

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ABSTRACT

Purpose: To assess whether the presence of an atherosclerotic lesion may alter the deposition kinetics of paclitaxel on the arterial wall after drug-eluting balloon (DEB) angioplasty, as well as paclitaxel concentrations in serum and in the recovered balloons.

Materials and Methods: Three New Zealand White rabbit models were created: an atheroma group (arterial mechanical injury and hyperlipidic diet; group A), a prelesional group (fat arterial infiltration, hyperlipidic diet; group B), and a control healthy group (group C). Forty-five animals underwent DEB angioplasty in the iliac artery. Arteries and serum samples were analyzed by liquid chromatography/tandem mass spectrometry at 1, 24, 48, 72, and 96 hours (arteries) and at 1, 6, 12, and 24 hours (serum). Recovered balloons were analyzed by UV chromatography. Histologic and statistical analyses were also performed.

Results: Group A showed significantly higher arterial paclitaxel concentrations in the first hour after DEB angioplasty (632.05 ng/mg vs 179.55 ng/mg in group A vs 1100645.64 and 168.54 ng/mg in groups B and C, respectively; \( P < .05 \)). Paclitaxel was undetectable in serum at 24 hours in all groups, but the amount was significantly higher (\( P < .05 \)) in group B at 1, 6, and 12 hours. The paclitaxel amount in navigated balloons from group A was significantly lower than in other groups (\( P < .05 \)).

Conclusions: Paclitaxel concentration in an atherosclerotic lesion model immediately after DEB angioplasty is nearly fourfold higher than in a healthy artery. Paclitaxel remains in the bloodstream longer when a universal state of fat arterial infiltration is achieved. These findings could have clinical implications, as studies testing commercial drug-eluting devices on healthy animals may be underestimating paclitaxel arterial uptake.

ABBREVIATIONS

DEB = drug-eluting balloon, DES = drug-eluting stent, HDL = high-density lipoprotein, LDL = low-density lipoprotein, TC = total cholesterol, TG = triglycerides

Drug-eluting stents and balloons have been developed to minimize intimal hyperplasia restenosis after angioplasty. They are used to deliver high concentrations of rapamycin, everolimus, or paclitaxel to the injured regions to inhibit vascular smooth muscle cell migration and proliferation, maintaining a patent lumen (1–4). Paclitaxel is considered the drug of choice in peripheral arteries. It interferes with the cell cycle predominantly at mitosis, disrupting microtubule dynamics by binding to \( \alpha \)-tubulin, resulting in the arrest of cells in the M phase of the cell cycle, leading to apoptosis (5). When delivered to the artery, paclitaxel causes cell cycle arrest of its smooth muscle and inhibits neointimal hyperplasia in experimental animals and humans (2,6,7).
Drug-eluting balloons (DEBs) have been developed to overcome the limitations of drug-eluting stents (DESs). However, a meta-analysis that compared the outcomes of DEB and DES use in the treatment of coronary artery lesions showed similarity in safety and clinical efficacy (8). When paclitaxel-coated balloon angioplasty was compared to uncoated balloon angioplasty in patients with femoropopliteal arterial disease, the former was superior to the latter in antstenotic efficacy (9–11). There are a lot of preclinical studies related to DESs but very few about DEBs, and most of them were developed in healthy experimental animals, with the exception of the study by Tzafriri et al (12), in which paclitaxel distribution was analyzed ex vivo in healthy and atherosclerotic aortas from rabbits and humans.

Experimental and clinical studies have described the accumulation, pharmacokinetics, and elution process of paclitaxel. Because of the strong affinity of paclitaxel for tubulin, paclitaxel is not homogenously distributed throughout the thickness of the arterial wall (1,13); consequently, this binding probably determines the diffusion and elution of the drug. Arteries do not have a homogenous structure, and the amount of muscular or elastic tissue differs depending on the anatomic territory. Finally, paclitaxel is a highly lipophilic compound that will bind more easily to a diseased atheromatous artery than to a healthy one, creating a different drug uptake by the diseased vessel (12,14,15).

It is likely that atheromatous plaques with associated disruption of the arterial wall architecture alter the elution kinetics of paclitaxel. In the present study, we compared paclitaxel elution kinetics among three rabbit models: normal iliac artery, arteriosclerotic artery, and fat-infiltrated artery.

**MATERIALS AND METHODS**

**Phase I: Development of Animal Models**

Fifty-seven male New Zealand White rabbits weighing 4–5 kg (age 7–8 mo) were randomly divided into three groups. The care and use of animals was in compliance with local animal welfare laws, guidelines, and policies. Our protocol was approved by the institutional review board ethics committee.

Three different models were created based on literature review (16,17): group A animals had atheroma lesion in the left iliac artery and hyperlipidemia, group B animals had a prelesional stage of fatty artery infiltration and hyperlipidemia, and group C was a healthy control group. The study design is summarized in **Figure 1**.

In animals from group A, an arterial injury was inflicted to the iliac arteries by using an angioplasty balloon before a 6-week hyperlipidic diet. Transcarotid catheterization was performed by using carotid artery cutdown and a 4-F introducer sheath (Micropuncture Introducer Set; Cook, Bloomington, Indiana). We obtained an aortoiliac arteriogram, and common iliac artery angioplasty was performed by using a 3-mm × 25-mm angioplasty balloon (Pantera; Biotronik, Berlin, Germany) inflated for 30 seconds at 7 atm three times. Postangioplasty iliac arteriograms were obtained in all animals (Fig E1, available online at www.jvir.org). The carotid artery was occluded, and animals were given

![Figure 1](images/f1.png)
enoxaparin (0.1 mL/24 h [each 24 h until euthanasia]; Clexane 40 mg; Sanofi-Aventis, Paris, France).

All animals from groups A and B were fed a hyperlipidic diet for 6 weeks (1% cholesterol, 10% sunflower meal, 6% palm oil; 2,400 kcal/kg; Centro de apoyo a la innovación, la investigación y la Transferencia de Tecnología, Valencia, Spain). Group C animals were fed with a standard rabbit diet (1,530 kcal/kg; Servicio de Experimentación Animal, Zaragoza, Spain). All animals had access to food at all times and were followed up daily to confirm correct diet intake. At the beginning of the study and every 2 weeks, blood samples were collected to study lipid profile, including total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL). After completion of the diet period, and because whole arteries were needed for the laboratory assays, nine animals were euthanized for the anatomopathologic study: seven from group A and two from group C. Euthanasia was performed by intravenous administration of sodium pentobarbital 120 mg/kg (Dolethal; Vétoquinol, Lure, France), and the left iliac artery, right iliac artery, and liver were sampled. They were cleaned and soaked in isopentane; all samples were embedded in Optimal Cutting Temperature medium. Histologic slices were cut by a Leica CM1510 S cryostat (Leica Microsystems, Nussloch, Germany) and stained with hematoxylin and eosin, elastin, and Oil Red.

**Phase II: Iliac DEB Angioplasty**

A single paclitaxel-eluting balloon angioplasty of the left common iliac and external iliac artery was performed in all remaining animals (17 in group A, 15 in group B, and 15 in group C), with a balloon inflation time of 30 seconds (3 × 25 mm, 3 μg/mm2; Pantera Lux; Biotronik). In all animals, iliac arteriograms were obtained before and after angioplasty. After angioplasty, the recovered balloons were cut off from the catheter to determine paclitaxel concentrations. Serial blood samples (2 mL) were taken immediately after angioplasty and at 1, 6, 12, and 24 hours (n = 3 animals at each time point). Animals were euthanized at different periods of time: 1, 24, 48, 72, and 96 hours, depending of the time point of artery sampling (n = 3 each). Arteries were carefully dissected, washed, weighed, and stored until assayed.

**Phase III: Measurement of Paclitaxel Concentrations in Blood, Iliac Artery, and Balloon after DEB Angioplasty**

Harvested arteries were crushed in a mortar with liquid nitrogen into powder and then dissolved into 3 mL of saline solution, with this step repeated twice. Liquid–liquid extraction was used for sample preparation, and docetaxel (Sigma-Aldrich, St. Louis, Missouri) was used as an internal standard. Serum samples (1 mL) were extracted the same way while avoiding the initial liquid nitrogen step.

For measurement of paclitaxel concentrations in the artery and serum, we used liquid chromatography/tandem mass spectrometry with a high-performance liquid chromatography system (Agilent 1100; Hewlett Packard, Palo Alto, California) and a spectrometer (Bruker Esquire 3000+; Bruker Daltonics, Billerica, Massachusetts) at a detection limit of 0.02 μg/mL.

The extraction method for balloons was slightly modified because of their higher paclitaxel concentrations. Paclitaxel concentration in balloons was assessed by a high-performance liquid chromatography method with UV detection at 220 nm. The detection limit of the assay was 0.5 μg/mL.

**Statistical Analysis**

To describe and plot the quantitative variables, we calculated the mean and the standard error of the mean. Normal distribution of quantitative variables was checked with the Kolmogorov–Smirnov test, discarding normal distribution when the P value was < .05. Parametric tests used were the Student t test for independent variables to compare two means and analysis of variance test to compare more than two means. As alternative nonparametric tests, we applied Mann–Whitney U and Kruskal–Wallis tests. In all cases of mean comparisons, we established an α-error of 0.05. Statistical analysis was carried out with SPSS software (version 17.0; SPSS, Chicago, Illinois).

**RESULTS**

**Phase I: Development of Animal Models**

There were no differences in feed consumption between the two diets. One animal died during the diet period and was replaced, and it was included in the total count (Materials and Methods). After 6 weeks of hyperlipidic diet (groups A and B), TC levels increased by 35–38 times versus baseline values, peaking at higher than 1,000 mg/dL, and showing highly significant differences versus basal levels taken on day 0 ($P < .001$; Fig 2). HDL and LDL levels also showed a significant increase ($P < .05$) compared with basal levels, but the difference in TG levels was not significant (135.24 mg/dL ± 54.37 day 0 vs 136.27 mg/dL ± 168.13). No significant differences were found between groups A and B at any week.

To assess the histopathologic models, nine animals were euthanized after completion of the diet period. All animals from group A (n = 7) showed varying degrees of liver steatosis. The following arteries were harvested: seven left iliac arteries from group A (A model), seven right iliac arteries from group A (B model), and four iliac arteries from group C (control). All animals that
had been receiving the hyperlipidic diet showed thickening of the arterial wall, disorganization and orientation change of smooth muscle cells, rupture of elastic fibers, and lipid infiltration in the neointima and media of the artery. However, arteries with a previous dilation lesion also showed varying degrees of luminal narrowing (Fig 3) and rupture of the internal elastic lamina with fat infiltration above and below it, whereas the others showed only fat deposits above the internal elastic lamina (Fig 4). None of the animals from group C showed liver steatosis or lipid artery infiltration.

**Phase II: Iliac DEB Angioplasty**

Two animals from group A died and had to be replaced to achieve 15 animals in each group for the laboratory assays. (The animals died in the second surgery, one related to the anesthetic procedure and the other as a result of blood loss at the carotid access site; these animals were included in the total count in Materials and Methods.) The rest of the animals did not have any problems related to the anesthetic or surgical procedures. In all animals, access to the left iliac artery through the carotid approach was feasible and balloon dilation was performed without complications.

**Phase III: Measurement of Paclitaxel Concentrations in Blood, Iliac Artery, and Balloon after DEB Angioplasty**

**Paclitaxel Concentrations in Blood.** Paclitaxel was detected in the blood after DEB angioplasty in all
animals. It showed gradually decreased concentrations over the period of 12 hours after DEB angioplasty and became undetectable at 24 hours. There were no differences between groups in blood paclitaxel levels at time 0 (an average of 0.30 μg/mL). At 1, 6, and 12 hours, we observed statistically significant differences between the groups, showing a higher blood paclitaxel concentration in group B (all \( P < .05 \)). All paclitaxel levels in blood are presented in Figure 5a.

**Paclitaxel Concentrations in Artery.** Figure 5b shows the evolution of paclitaxel concentrations in the common iliac arteries. Group A showed a significantly higher concentration of paclitaxel in artery at 1 hour and 24 hours, approximately four times the concentrations in the other groups (632.05 ng/mg ± 125.75 in group A vs 179.55 ng/mg ± 45.64 in group B and 168.54 ng/mg ± 83.48 ng/mg in group C; \( P < .05 \)). After that, at 48, 72, and 96 hours, that difference was not significant among groups. Group C showed the lowest concentration of paclitaxel in the artery at all times and was below the detection limit of the assay at 96 hours. In groups A and B, paclitaxel was still present in the arterial wall at 96 hours (2.53 ng/mg ± 1.58 and 1.72 ng/mg ± 1.97, respectively).

**Paclitaxel Amounts in DEBs.** There were significant differences between the paclitaxel amounts recovered from group A navigated balloons (84.62 μg ± 7.32) and those from the other groups (113.93 μg ± 10.69 in group B and 97.60 μg ± 11.54 in group C; \( P < .05 \)).

**DISCUSSION**

Despite insufficient information on the pharmacokinetics of paclitaxel DEBs, several different devices are successfully used in clinical practice, mainly in two presentations—3 μg/mm² (10) and 2 μg/mm² (11)—with various excipients. Most of the animal studies of paclitaxel DEB angioplasty have been conducted in animal models with normal healthy arteries. In addition, the concentrations of paclitaxel in normal and atheromatous arteries, balloons, and blood after DEB angioplasty are largely unknown. Several authors have measured free paclitaxel and its uptake by the arterial walls (1,2,12,18), concluding that wall architecture may play an important role in the deposition and pharmacokinetics of the drug. Tzafriri et al (12) analyzed paclitaxel distribution into the wall in rabbit and human aortas, in both healthy and atherosclerotic arteries, in an in vitro experiment. In the present in vivo study, we measured the paclitaxel delivered by DEB angioplasty in blood and arterial wall in healthy and atherosclerotic rabbit models. Herdeg et al (19) and Axel et al (20) have also carried out similar studies in vivo, but with drug infusion catheters instead of DEB devices.
In the present study, rabbits were chosen as an animal model for a pharmacokinetic study of paclitaxel of DEBs because the animals are known to develop hypercholesterolemia via accumulation of exogenous cholesterol when fed with a high-cholesterol diet (21). Rabbits are considered hypercholesterolemic when the levels of plasma total cholesterol are greater than 1,000 mg/dL (22), and we achieved this level with a hyperlipidic diet, showing significant differences in TC, HDL, and LDL levels. TG concentration differences between groups were not significant, probably because blood TG levels are very dependent on the time of fat intake, and our animals had food available at all times, which resulted in a high standard deviation. The histopathologic study supported the success in creating an atherosclerotic model, similar to stages II, III (ie, prelesional stages, group B), and IV (group A) of human atherosclerosis (23) and not achieving a well-defined fibrous cap or calcification or rupture of the plaque in any of the animals (stages V and VI) because of the short evolution of the disease compared with that seen in humans (24).

In the present study, paclitaxel concentrations were measured in blood, harvested arteries, and recovered DEBs by chromatography. In our experience, atheromatous-like lesions as well as fatty infiltration of the arteries modified the pharmacokinetics of paclitaxel. As in other studies (25), the highest concentration in serum was achieved just after balloon angioplasty and was similar in all groups, with a gradual decrease to undetectable levels at 24 hours. At 1, 6, and 12 hours, serum paclitaxel concentration was higher in group B, showing a slower distribution and/or elimination, whereas arterial wall paclitaxel concentration was always higher in group A. In recovered DEBs from group A, paclitaxel concentrations were significantly lower than in recovered balloons from groups B and C, which can be explained by the increased initial drug uptake by the injured arterial wall.

One hour after DEB angioplasty, paclitaxel concentration in arterial wall in group A was nearly fourfold higher than in groups B and C. At 96 hours, arterial wall paclitaxel levels were below the detection limit in the control group, but still detectable in groups A and B. The healthy artery concentrations shown in the present study are similar to those in other works, such as the study of Granada et al (26), and higher than therapeutic levels (26). As in similar studies, short-term arterial levels of paclitaxel following DEB angioplasty are much higher compared to DESs, but the toxicity level for smooth muscle and endothelial cells is not yet known (27), being suggested a threshold of 100 ng/mg (26). However, controlled clinical trials (9–11) showed no adverse consequences of this temporary overdose.

The present work shows that data extracted from healthy animals is probably underestimated, so the paclitaxel concentration in atheromatous arteries during the first hours may be even higher than initially supposed.
Atherosclerotic artery walls (group A) seem to take up a significantly larger amount of paclitaxel from the delivery balloon, whereas in prelesional stages of fatty infiltration (group B), this uptake is not marked, but paclitaxel remains in the bloodstream longer, probably because of its lipophilicity. These results are in agreement with those of Tzafiri et al. (12), who stated that paclitaxel deposition is highly dependent on wall architecture and composition (higher in elastin and microtubule-rich areas), increasing its deposition in balloon-injured areas (group A). However, lipid infiltration alone does not increase significantly wall uptake, as, according to these authors (12), this produces a concomitant reduction of \( \beta \)-tubulin and elastin in these areas.

One of the most important limitations of the present study is that an experimental rabbit model does not faithfully reproduce the last stages of arteriosclerotic disease in humans, although it still is a good pathophysiological model and superior to a healthy model from a pharmacokinetic point of view. In addition to that, extrapolation of animal data to humans might not always be linear. The limit of detection of our experiments was too high to obtain long-term data (ie, weeks, months). Finally, there is a lack of studies in pathologic rabbit iliac artery to which to compare the present results.

The presence of an atheromatous lesion in the arterial wall plays an important role in drug uptake, increasing the amount of paclitaxel deposition compared with healthy models. This may have a repercussion in drug calculation for commercial balloons, which are routinely tested in healthy models, because, even though several controlled randomized studies (8–11) have shown efficacy and safety of these devices, the dosage may be underestimated when used in real lesions. We believe similar studies and more immunohistochemical analyses are needed to assess these results and give more evidence to guide the use of drug-eluting devices, which seems poised to become a stronghold of endovascular interventions in the near future.

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**REFERENCES**


Figure E1. Fluoroscopic images. (a) Control iliac angiography shows a patent distal abdominal aorta and iliac arteries. (b) A 3-mm × 25-mm angioplasty balloon catheter is navigated to the left common and external iliac arteries. (c) Balloon inflation. (d) Angiography after angioplasty shows proximal arterial dilation and distal vasospasm.