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Experimental assessment of effects of antiproliferative drugs of drug-eluting stents on endothelial cells☆

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ABSTRACT

Background: Late and very late stent thrombosis after drug-eluting stent implantation is a major concern. The present study evaluated difference in the effects of sirolimus, paclitaxel and zotarolimus on endothelial cells.
Methods: Mouse endothelial cells were seeded in a 6-well plate. Cells were cultured with an antiproliferative drug at the expected concentrations for each well for 24 hours before making 3 scratch lines with a pipette tip. After a 4.5 hour incubation period, 3 reference scratch lines, vertically across the original scratch lines, were made in the same way. The experiment was repeated at least 6 times (6 plates). Measurements were performed at 9 crossings of each well. Wound healing ratio was calculated as 1 − (distance of the first scratch/distance of the second scratch). % cell migration was calculated as (wound healing ratio at an expected drug concentration/wound healing ratio with no drug) × 100. Average % cell migration at 54 crossings of 6 plates was calculated.
Results: Paclitaxel inhibited cell migration in a concentration-dependent manner. On the other hand, concentration-dependent inhibition was not observed for sirolimus or zotarolimus. Sirolimus showed a stronger inhibitory effect on migration of endothelial cells compared to zotarolimus.
Conclusions: The difference in the effect of antiproliferative drugs of drug-eluting stents on endothelial cells may be associated with relatively faster re-endothelialization of zotarolimus-eluting stent compared to the 1st generation DES.

1. Introduction

Drug-eluting stent (DES) has dramatically reduced in-stent restenosis. However, late and very late stent thrombosis after DES implantation has emerged as a major concern. The antiproliferative drugs used in DES not only inhibit intimal hyperplasia but also delay re-endothelialization. Recent trials have shown a lower stent thrombosis rate of the 2nd generation DES compared to the 1st generation DES [1]. Thus the effect of the antiproliferative drugs of the 1st and 2nd generation DES on re-endothelialization may be different. The present experimental study evaluated difference in the effect of the antiproliferative drugs of the 1st and 2nd generation DES on endothelial cells.

2. Methods

2.1. Chemicals

Chemicals were purchased from the following commercial sources: paclitaxel (Wako Chemicals, Japan), zotarolimus (Toronto Research Chemicals, Canada), and rapamycin (Sigma, USA). Ethanol was used as solvent for paclitaxel and rapamycin, and DMSO for zotarolimus.

2.2. Cell culture

Mouse vascular endothelial cell line, UV♀2 (RCB1994) was provided by the RIKEN BRC through the National Bio-Resource Project of the MEXT, Japan. Cells were cultured at 37 °C in a 5% CO_2, humidified atmosphere. Cells were grown in Dulbecco's Modified Eagle's Medium (High glucose, Sigma, USA) with 10% fetal bovine serum (HyClone, USA), 100 units/ml penicillin, and 100 μg/ml streptomycin from Nacalai Tesque (Kyoto, Japan).

2.3. Scratch assay

We assessed the effects of antiproliferative drugs of DES on cultured endothelial cells by scratch wound assay. Cells were seeded at the density of 0.7–1.5 × 10^5 cells/well in a 6-well plate. Following the preculture period for 24 hours (Fig. 1), the volume of culture medium in each well was adjusted to 2 ml to start the incubation of cells with a drug at the expected concentrations for each well in the plate. The drug concentrations were selected according to those in previous studies [2,3]. Cells were cultured for another 24 hours before making 3 scratch lines with a pipette tip (200-μl size), and the closure of the scratch lines was measured after a 4.5-hour incubation period. Three reference scratch lines, vertically across the original scratch lines, were

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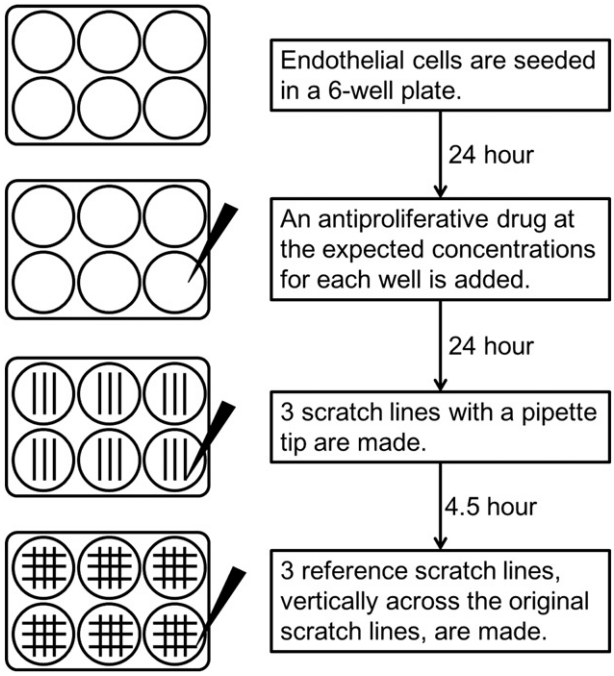

Fig. 1. Experimental flow chart.

made in the same way just before fixing cells with 4% paraformaldehyde for 10 min at room temperature prior to taking images under the microscope at 40× magnification (Fig. 2) [4]. The images acquired for each sample were analyzed quantitatively by using ImageJ 1.48v (NIH, USA). The experiment was repeated at least 6 times (6 plates).

Cell migration was evaluated by comparing the images between the solvent-treated control cells and the drug-treated cells. For each image, measurements were performed at 9 crossings of each well (Fig. 1). The distances of the first scratch and the second scratch were measured at 6 points (Fig. 2). The mean distances of the first scratch and the second scratch were calculated. Wound healing ratio was calculated as 1 − (mean distance of the first scratch/mean distance of the second scratch). % cell migration was calculated as (wound healing ratio at an expected drug concentration/wound healing ratio with no drug) × 100. Average % cell migration at 54 crossings of 6 plates was calculated.

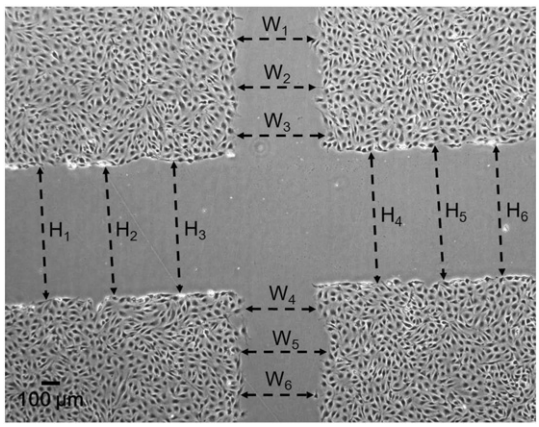

Fig. 2. Measurements of cell migration. The distances of the first scratch line (W1-6) and the second scratch line (H1-6) are measured at 6 points. The mean distances of the first scratch and the second scratch are calculated. Wound healing ratio was calculated as 1 − (mean distance of the first scratch/mean distance of the second scratch).

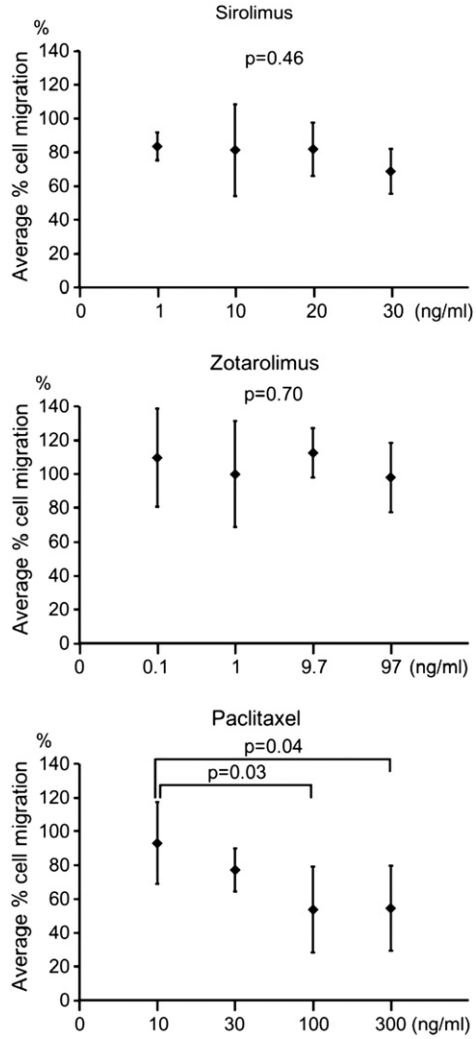

Fig. 3. The effect of antiproliferative drugs on endothelial cell migration. Paclitaxel inhibits cell migration in a concentration-dependent manner. On the other hand, concentration-dependent inhibition is not observed for sirolimus or zotarolimus.

2.4. Statistical analysis

Statistical analysis was performed with SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Values are shown as mean ± SD. Continuous variables were compared using the Student's t test or one-way analysis of variance. Statistical significance was defined as $p < 0.05$

3. Results

Paclitaxel inhibited cell migration in a concentration-dependent manner (Fig. 3). On the other hand, concentration-dependent inhibition was not observed for sirolimus or zotarolimus. Pharmacokinetics of sirolimus-eluting stent and zotarolimus-eluting stent were reported [5,6]. However, there is little information about pharmacokinetics of paclitaxel-eluting stent. Thus we compared the effect of sirolimus and zotarolimus at the concentrations that were closest but higher than the maximum concentration after sirolimus- (0.86 ± 0.21 ng/ml) and zotarolimus-eluting stent implantation (1.80 ± 0.53 ng/ml) [6]. Sirolimus showed a stronger inhibitory effect on migration of endothelial cells compared to zotarolimus (Fig. 4), although concentration of zotarolimus compared to that after stent implantation was much higher than that of sirolimus.