Sonodynamic Therapy Decreased Neointimal Hyperplasia After Stenting in the Rabbit Iliac Artery

Koh Arakawa, MD; Kousukue Hagisawa, MD; Hiroyuki Kusano, MD; Satoru Yoneyama, MD; Akira Kurita, MD; Tsunenori Arai, PhD; Makoto Kikuchi, PhD; Isao Sakata, PhD; Shin-ichirou Umenura, PhD; Fumitaka Ohsuzu, MD

Background—In-stent restenosis remains a pivotal problem after coronary and peripheral stenting. Sonodynamic therapy inhibits tumor growth by means of cytotoxicity after the activation of sonochemical sensitizers by ultrasound. PAD-S31 is known to be a water-soluble, chlorin-derivative sonochemical sensitizer. We assessed the efficacy of sonodynamic therapy using this sensitizer on neointimal hyperplasia in a rabbit stent model.

Methods and Results—Stents were implanted in the iliac arteries of 16 rabbits. A total of 32 stented arteries were randomized to sonodynamic therapy, control, ultrasound exposure, and PAD-S31 groups. One hour after the intravenous administration of PAD-S31 (25 mg/kg body weight), ultrasound energy (1 MHz, 0.3 W/cm²) was delivered transdermally to the sonodynamic therapy group. At 28 days, all stent sites were analyzed morphometrically. The size of the intimal cross-sectional area was smaller in the sonodynamic therapy group than in the control, ultrasound, and PAD-S31 groups (0.31 ± 0.07 versus 1.38 ± 0.47, 1.66 ± 0.71, and 1.61 ± 0.42 mm², respectively; P < 0.05). The ratio of the intimal and medial cross-sectional area was smaller in the sonodynamic therapy group than in the control, ultrasound, and PAD-S31 groups (0.71 ± 0.22 versus 2.53 ± 1.39, 2.48 ± 0.60, and 3.45 ± 1.42 mm²; P < 0.05).

Conclusions—Sonodynamic therapy with PAD-S31 is considered to be a feasible treatment modality for noninvasively inhibiting neointimal hyperplasia in a rabbit iliac stent model. (Circulation. 2002;105:149-151.)

Key Words: ultrasonics ▪ stents ▪ restenosis ▪ sonodynamic therapy

Use of stent implantation has improved the restenosis rate after balloon angioplasty. In-stent restenosis (ISR), however, remains a pivotal limitation after coronary and peripheral stenting. Both neointimal hyperplasia and matrix production, which are initiated by activated smooth muscle in response to vessel wall injury, have been shown to play an important role in ISR.

A water-soluble chlorin derivative, 13,17-bis[1-carboxypropionyl] carbamoyl-l-3-ethyl-8-ethoxyiminomethylene-7-hydroxy-2,7,12,18-tetramethyl porphyrin sodium (PAD-S31), which is the analogue of a new photosensitizer (ATX-S10), has the same biological effect as that of ATX-S10 for tumor treatment,5 and it also is known as a sonochemical sensitizer.6 A new treatment modality called sonodynamic therapy (SDT) has been developed, in which the ultrasound-induced cytotoxicity of sonochemical sensitizers inhibits tumor growth. The administration of PAD-S31 followed by ultrasound exposure has been shown to inhibit tumor growth both in vitro and in vivo. As a result, the sonodynamic effect of PAD-S31 on ISR was investigated in a rabbit model.

Methods

Animal Preparation and Stent Implantation

Japanese white rabbits (3 kg weight; n = 16, Tla, Tokyo) were fed a 1% cholesterol diet containing 100 mg of aspirin per 100 g of diet 1 week before stent implantation. The diet was continued for 4 weeks after stenting. The rabbits were preanesthetized with xylazine (3 mg/kg) and ketamine (20 mg/kg) intramuscularly and maintained with pentobarbital intravenously. After cutting the skin, the femoral artery was exposed. A 9-mm stent (Wiktor) was remounted on a balloon catheter measuring 3 mm in diameter and 20 mm in length when inflated. After administering 200 U/kg of heparin, the stent was delivered into the common iliac artery with 6 atmospheres of inflated pressure. A total of 32 stents were implanted, consisting of 8 stents for the control group, 8 for the ultrasound exposure group, 8 for the PAD-S31 group, and 8 for the group that received ultrasonic exposure with PAD-S31 (SDT). All rabbits were killed at 28 days after stent implantation.

Sonodynamic Therapy

An ultrasonic transducer measuring 24 mm in diameter (Hitachi Central Research Laboratory) was operated at 1 MHz. The ultrasonic intensity output from the transducer was calibrated against the drive voltage in water in a progressive wave mode. A water bag was placed on the body and the transducer was put on the water bag above the stent under fluoroscopic guidance. The transducer was driven at a voltage level corresponding to the calibrated ultrasonic output intensity of 0.3 W/cm². The ultrasonic energy was delivered transdermally through the water bag to the stented artery for 15 minutes. As a sonodynamically active agent, 25 mg/kg of PAD-S31 (Photochemical Co, Ltd) was administered intravenously 1 hour after stent implantation.
before ultrasonic exposure, on the basis of the finding of a previous study that showed the maximum tissue concentration to be achieved at 1 hour after the intravenous administration in normal tissue. In 2 rabbits, a thermistor probe (Needle probe, T/T-T30, Omega) was placed in the subcutaneous tissue between the stent and the ultrasound transducer, and the tissue temperature was measured with an analyzing recorder (AR1100A, Yokogawa).

**Pathological Studies**

The stented arteries were excised and fixed with 10% formalin and embedded in polyester resin (Rigolac 2004 and 70F, Showa High-polymer Co, Ltd). Each block of the stented artery was divided into 3 parts: both ends and a middle part. Each part was cut with a crystal cutter at 3- to 500-μm widths and then ground (Speed Lap ML-521-d and HT, Maruto Instrument Co, Ltd) to a thickness of 80 μm. Each section was stained with van Gieson. The narrowest part of the stented artery was evaluated as a representative part. The lumen cross-sectional area (CSA), vessel CSA, intimal CSA, and injury score were all measured by a pathologist (H.K.) who did not know the treatment category. The percent area stenosis was determined as (vessel CSA − lumen CSA)/vessel CSA.

**Statistical Analysis**

The data are presented as the mean±SD. Any differences between the individual groups were tested by 1-way ANOVA, and the significance of differences in unpaired measurement was assessed by the Bonferroni t test. P<0.05 was considered to be statistically significant.

**Results**

All rabbits tolerated the SDT treatment well and no skin erosion or skeletal muscle damage at the treated site was observed during the 28-day follow-up. Morphometrical analysis was done 28 days after stenting. Representative cross-sections for the control, ultrasound exposure, PAD-S31, and SDT groups are shown in Figure 1. The arteries treated with SDT showed mild neointimal hyperplasia, whereas the control, ultrasound exposure, and PAD-S31 arteries all showed moderately increased neointimal hyperplasia. No significant difference was seen in the injury scores among the control, ultrasound, PAD-S31, or SDT groups (1.8±0.4, 1.6±0.4, 1.7±0.2, and 1.7±0.4). The lumen CSA (Figure 2) was significantly larger in the SDT group (ANOVA, P<0.01) than in the control group (2.75±0.74 versus 1.43±0.39 mm²; P<0.05), but not larger than in the ultrasound and PAD-S31 groups (2.07±0.82 and 2.45±0.45 mm²). The

![Figure 1](image-url)  
**Figure 1.** Histological cross-sections of a stented segment in the rabbit iliac artery. Neointimal growth occurred in the control (A), ultrasound exposure (B), and PAD-S31 (C) groups. The degree of neointimal growth was less in the SDT group (D).

![Figure 2](image-url)  
**Figure 2.** The lumen, intimal, medial, and vessel CSA, ratio of the intimal and medial CSA, and percent area stenosis at 28 days for the control (Cont), ultrasound exposure (US), PAD-S31 (PAD), and sonodynamic therapy (SDT) groups. * P<0.05 versus Cont, † P<0.05 versus US, ‡ P<0.05 versus PAD.
intimal CSA was significantly smaller in the SDT group (ANOVA, \(P<0.001\)) than in the control, ultrasound, and PAD-S31 groups (0.31±0.07 versus 1.38±0.47, 1.66±0.71, and 1.61±0.42 \(\text{mm}^2\); \(P<0.05\)). The ratio of intimal and medial CSA was also smaller in the SDT group (ANOVA, \(P<0.001\)) than in the control, ultrasound, and PAD-S31 groups (0.71±0.22 versus 2.53±1.39, 2.48±0.60, and 3.45±1.42 \(\text{mm}^2\); \(P<0.05\)). In addition, no differences were seen regarding the medial CSA in any of the 4 groups. The percent area stenosis was smaller in the SDT group (ANOVA, \(P<0.001\)) than in the control, ultrasound, and PAD-S31 groups (23±7% versus 58±11%, 53±14%, and 65±9%; \(P<0.05\)). The histological findings of the arteries treated with SDT seemed to be essentially normal. The tissue temperature during ultrasound exposure was measured in 2 rabbits undergoing SDT. The mean change in the temperature was +1.4±1.3\(^\circ\)C.

**Discussion**

In the present study, we demonstrated that SDT with PAD-S31 reduced the degree of intimal hyperplasia after stent implantation in the rabbit iliac artery, by the intravenous administration of sonochemical sensitizer and transdermal ultrasound exposure. Mechanism for the activation of a sensitizer by ultrasound is still not well understood. There are 2 possibilities,\(^5\)\(^9\)\(^10\): namely: (1) Ultrasound energy itself activates a sonochemical sensitizer, or (2) acoustic cavitation induces light emission (sonoluminescence), which thus activates the sonochemical sensitizer. The spectrum of sonoluminescence\(^11\) is broad in wavelength, from 200 nm to 700 nm, with the most intense emission at \(\sim 300\) to 400 nm. The most intense absorbance of PAD-S31 is \(\sim 400\) nm in wavelength, which is \(\sim 4\) times greater than that at a second peak absorbance of 670 nm. A shorter wavelength of light has less transmission in tissue depth than a longer wavelength has. Therefore, despite the low activation efficiency of 670 nm, coherent light, a longer wavelength of light,\(^12\) must be used to clinically activate the sensitizer in photodynamic therapy (PDT). On the other hand, ultrasound has an appropriate tissue penetration, no matter which mechanism activated the sensitizer.

PDT is an intriguing new approach for inhibiting injury-induced intimal hyperplasia.\(^11\) An activated sensitizer induced cell depletion, while inhibiting both cell growth and cytokine release and thereby inhibiting the development of intimal hyperplasia.\(^13\)\(^–\)\(^15\) Ultrasoundically activated PAD-S31 reduced the degree of intimal hyperplasia without medial mass reduction. Interestingly, the cell nuclei counts in the media did not seem to decrease and no acellular media was observed in the SDT group.

Recently, sonotherapy\(^16\) was introduced to prevent ISR both experimentally and clinically. In our study, SDT inhibited neointimal hyperplasia after stenting more effectively than ultrasound alone. Ultrasound itself had no effect on the inhibition of neointimal hyperplasia because the ultrasound power we used in this study was insufficient for sonotherapy. However, the ultrasound power was strong enough to have a sonochemical effect on PAD-S31. The different ultrasound used in our study (operating mode of continuous wave versus pulse, frequency of 1 MHz versus 700 KHz, exposure time of 15 minutes versus 2 minutes) may also have played a role in the better results of SDT.

**Limitations**

The number of rabbits studied in each group was relatively small and the follow-up period was only 28 days. We studied only one condition of ultrasound (1 MHz, 0.3 W/cm\(^2\)) and sonochemical sensitizer (25 mg/kg of PAD-S31). This dose\(^4\) was the same as that used for tumor treatment with SDT in an animal model. This treatment has the potential to be clinically adopted; however, further studies elucidating the dose-response of the ultrasound output and sonochemical sensitizer and the long-term effect are still called for.

**Conclusions**

These preliminary data suggest that SDT with PAD-S31 be considered a feasible treatment modality for noninvasively inhibiting neointimal hyperplasia in a rabbit iliac stent model. However, the exact mechanism of SDT on the vessel wall and the optimal conditions for using the ultrasound output and sonochemical sensitizer still remain to be elucidated in future studies.

**References**

Sonodynamic Therapy Decreased Neointimal Hyperplasia After Stenting in the Rabbit Iliac Artery

_Circulation_. 2002;105:149-151
doi: 10.1161/hc0202.102921
_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2002 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/105/2/149

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org//subscriptions/