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EVALUATION OF LOCAL PLATELET DEPOSITION DURING LASER THERMAL ANGIOPLASTY

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INDEXING WORDS

platelet deposition; laser angioplasty; Nd:YAG laser; metal tip probe

SYNOPSIS

Laser thermal angioplasty is one of the brand-new transluminal interventions for arterial occlusive disease. And one of the most important prognostic factors of this intervention is the degree of local platelet deposition which causes the acute platelet thrombus and increases the proliferation of smooth muscle cells. The purpose of this study is to assess the degree of platelet deposition on the laser ablated area and to investigate the optimal conditions of laser ablation from the point of thrombogenesis.

First of all, the laser ablations of various delivered energy were carried out on canine femoral arteries, then thrombus formation after laser ablation was evaluated with angioscopy. No thrombus was recognized on all ablated areas in the cases with a laser energy of 16 Joule (J) and 20 J. On the other hand, small thrombus on the ablated area was observed in 1 of 4 cases (25%) with that of 24 J, and in 2 of 4 cases (50%) with that of 30 J.

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Then, the degree of platelet deposition on the laser ablated area was evaluated with platelet labeled radioimmunoassay. Laser ablations were employed in canine femoral arteries varying delivered laser energy. There were no differences in the numbers of platelet deposition when the laser energy for one shot was within 24 J. The numbers of platelet deposition with a laser energy of 30 J and 45 J were significantly ($p<0.05$) higher than those with a laser energy within 24 J. The numbers of platelet deposition were gradually increased related to the frequency of laser ablations, and the numbers of platelet depositions in 10 repeated laser ablations of 16J and 20J were significantly ($p<0.05$) higher than that in single laser ablation of 16J and 20J.

From the aspect of local platelet deposition, the optimal laser energy for one shot should be restricted within 24 J and the repeated ablation was the most appropriate method of laser thermal ablation.

INTRODUCTION

Laser thermal angioplasty is one of the recent developed transluminal interventions for arterial occlusive diseases. Excellent clinical results have been reported,[17,20] and it has become one of the effective and less-invasive treatments for atherosclerotic diseases, especially in peripheral arteries. One of the most important prognostic factors of this intervention is the degree of local platelet deposition which cause the acute platelet thrombus and increase the proliferation of smooth muscle cells. Several experimental outcomes concerning about platelet deposition after laser angioplasty were reported previously,[1,12,16] however, it has not been yet cleared the relationship between delivered laser energy and platelet deposition.

The purpose of this study is to assess the quantity of platelet deposition on the laser ablated area of the arterial wall, and to investigate the optimal conditions of laser ablation from the point of thrombogenesis.

MATERIALS AND METHODS

All animal experiments were conducted according to the "Guideline for Animal Experimentation at Kobe University School of Medicine".

Laser delivery and probe

A Neodymium-yttrium aluminum garnet laser (Nd:YAG laser) was generated by

MEDILASER-YAG 500 (model MEL-50, Mochida Pharmaceutical Co., Tokyo, Japan) and transmitted to the metal tip of 2.5 mm in diameter through a 0.8 mm core quartz fiber (metal tip probe: hereafter MTP(metal tip probe), HGM Inc., Salt Lake City, UT, USA).

Experiment I:

Experimental procedure (*Fig. 1*)

First of all, thrombus formation after laser ablation was evaluated with angioscopy. Four mongrel dogs weighing between 10-15kg were used. After premedication with ketamine hydrochloride (2mg/kg, intramuscularly), the animals were anesthetized with thiamylal sodium (10mg/kg, intravenously), intubated and allowed to ventilate spontaneously on room air. Anesthesia was maintained with intermittent administration of pentobarbiturate. The electrocardiogram and arterial blood pressure were continuously monitored throughout the procedure. Bilateral femoral arteries were surgically exposed. After intravenous administration of heparin sulfate (100 IU/kg), a 10 Fr. sheath catheter was introduced through a right carotid cutdown and placed in the descending aorta. Another 10 Fr. catheter was introduced through the distal side of the femoral artery. The MTP was advanced through the catheter into both femoral artery with retrograde fashion. Then, laser ablations were performed on the inner surface of the femoral artery with the following four different conditions; 8 watts (W)×2 seconds (16 Joule: J), 10 W×2 seconds (20 J), 8 W×3 seconds (24 J), 10 W×3 seconds (30 J).

Angioscopic examination

Three hours after laser ablations, an angioscopic fiber of 0.75 mm in diameter (AS-001, Fukuda Electric Co.,Tokyo, Japan) was introduced to the femoral artery with the occlusion balloon catheter through the sheath catheter which placed in the descending aorta. The angioscopic fiber was connected to a fiber imaging system (FV-3000, Medical Science Co., Tokyo, Japan), and the images were observed on a video monitor. The laser ablated arteries were observed angioscopically with a gentle flush of saline.

Experiment II:

Experimental procedure (*Fig. 2*)

Seven mongrel dogs (13 femoral arteries) weighing between 9-16 kg were used. The dogs were anesthetized in the same manner described above. After intravenous administration of heparin sulfate (100 IU/kg), a 10 Fr. sheath catheter was introduced through a right carotid cutdown and the tip of the catheter was placed in the descending aorta. The MTP was advanced through the catheter into both femoral arteries under fluoroscopic guidance. Laser ablations were performed starting from the distal end of the femoral arteries. Each ablation was done in a 1 cm segment of the femoral arteries, and the MTP was moved back and forward at the speed of 2cm/sec during ablation.

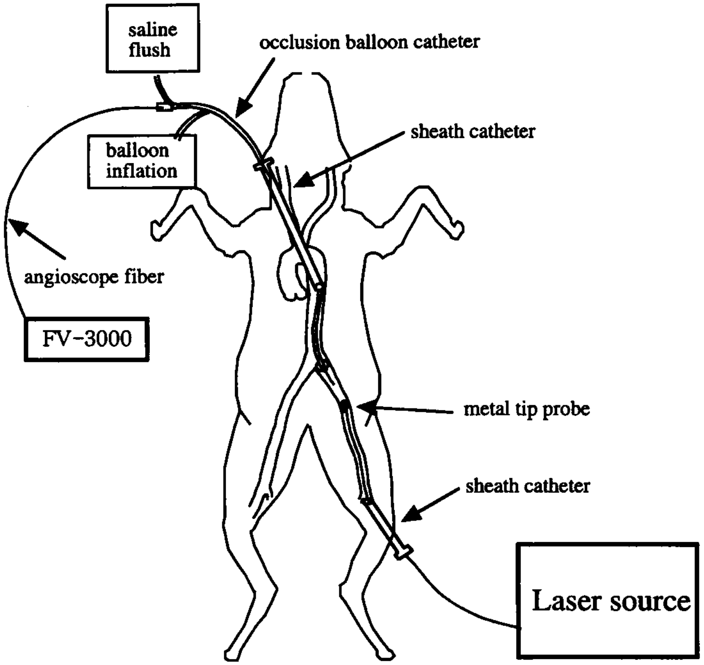

Fig. 1. The schema of laser ablation and angioscopic observation in Experiment I.

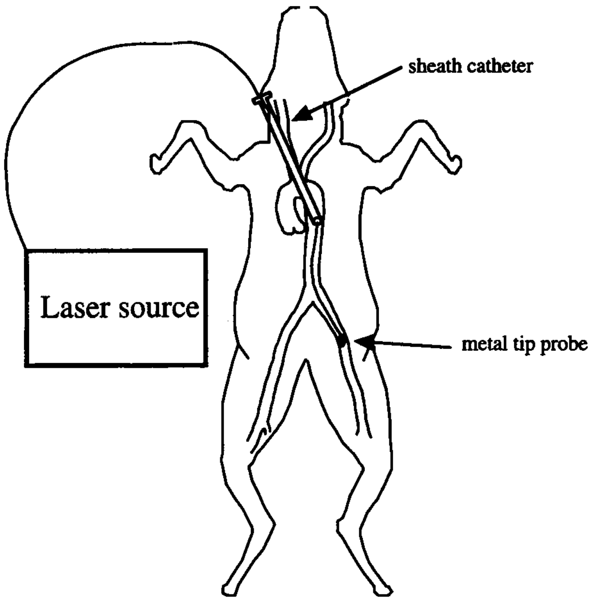

Fig. 2. The schema of laser ablation in Experiment II.

Two methods of laser ablations were employed varying delivered laser power and duration of ablation. That is, 1) single ablation of 8 W×2 seconds (16 J), 10 W×2 seconds (20 J), 8 W×3 seconds (24 J), 10 W×3 seconds (30 J), and 15 W×3 seconds (45 J), 2) Three, 5 and 10 times repeated ablations of 8 W×2 seconds (16 J) and 10 W×2 seconds (20 J).

Three hours after laser ablations, the dogs were sacrificed with over dose pentobarbiturate infusion. Both the femoral arteries were excised after gentle perfusion with saline solution. Each ablated lesions were divided and radioactivity of the specimen was determined with the gamma counter. The left carotid artery was excised with same manner and used as a control.

Quantification of platelet deposits

Autologous blood of 40 ml was obtained from the catheter inserted via the right carotid artery and was anticoagulated with 6ml of acid dextrose solution (ACD solution) and then centrifuged at 200 G for 10 minutes. The supernatant platelet-rich plasma (PRP) was transferred to another tube. The PRP was centrifuged at 1000 G for 10 minutes to form a platelet pellet. The supernatant platelet-poor plasma (PPP) was removed and platelet pellet washed once by overlaying with 20 ml of ACD solution (pH 6.5) to remove residual plasma proteins. The pellet was then resuspended in 1ml of ACD solution (pH 6.5). Ten to thirty-sevene Mbq ^{111}Indium oxine (Amersham international plc. UK) was added and incubated for 10 minutes at 37℃. After incubation , unbound ^{111}Indium oxine was removed by washing with ACD solution (pH 6.5). Labeling platelets were resuspended in 5 ml of PPP and reinjected. Mean labeling efficiency was 93%±3% (mean±SD).

The labeling platelets were reinjected just after laser ablation. Shortly before the death of the animals, 2 ml of blood was obtained to determine the mean radioactive counts with a gamma well counter (1470 WIZARD™ Pharmacia Biotech). Another 2 ml of blood were collected in two EDTA tubes to calculate the mean platelet counts/ml blood. Ablated arteries were excised and radioactivity was determined with a gamma counter. The number of the platelets/ml blood were divided by the ^{111}Indium counts per 2 minutes (cp2m)/ml blood. The numbers of platelets/mm^2 arterial segment were calculated by multiplication of the ^{111}Indium cp2m/ mm^2 arterial segment with the number of platelets /^{111}Indium cp2m.

Statistical analysis

The results were expressed as mean values ± standard deviation (S.D.). Platelet depositions were compared by analysis of variance (ANOVA). A p-value of <0.05 was evaluated as a significant value.

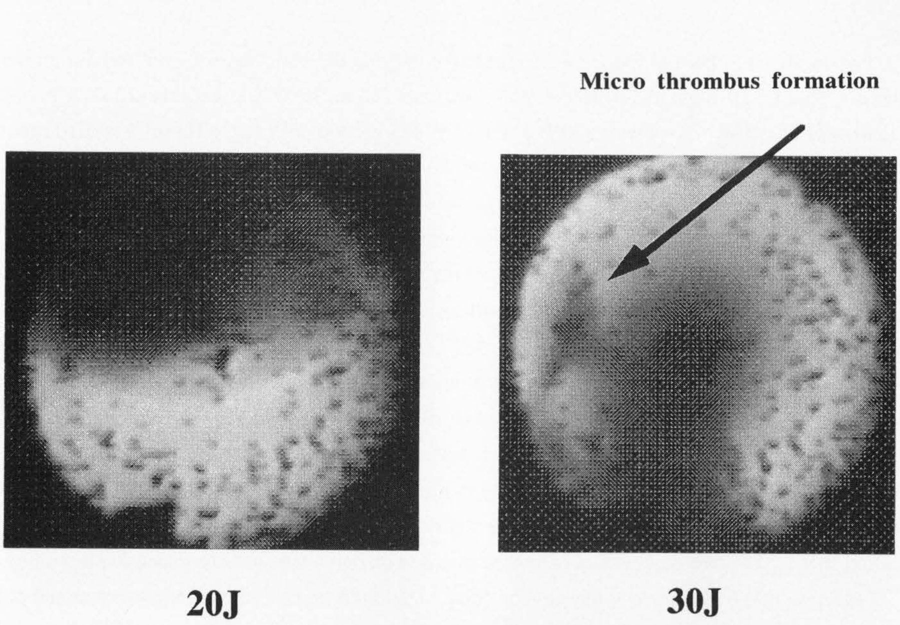

Fig.3. Angioscopic evaluation 3 hours after laser ablation.

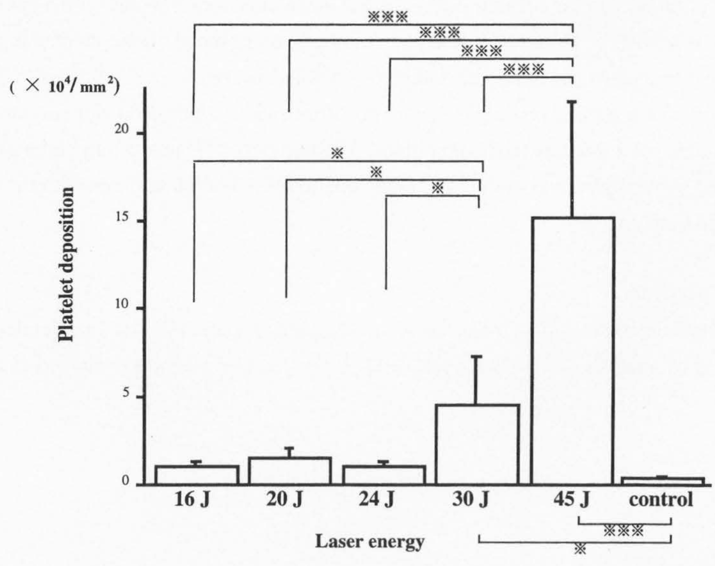

Fig. 4. The number of the platelet deposition after single laser ablation.
※ $P<0.05$ ※※※ $P<0.001$

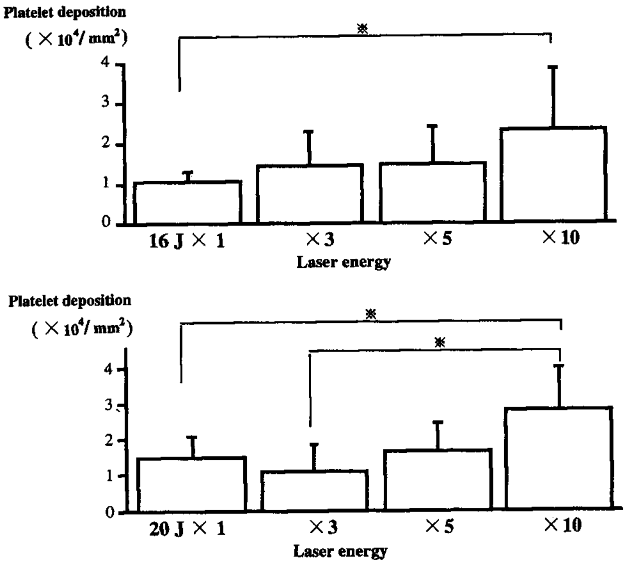

Fig. 5. The number of the plaelet deposition after repeated laser ablation.
※ $P<0.05$

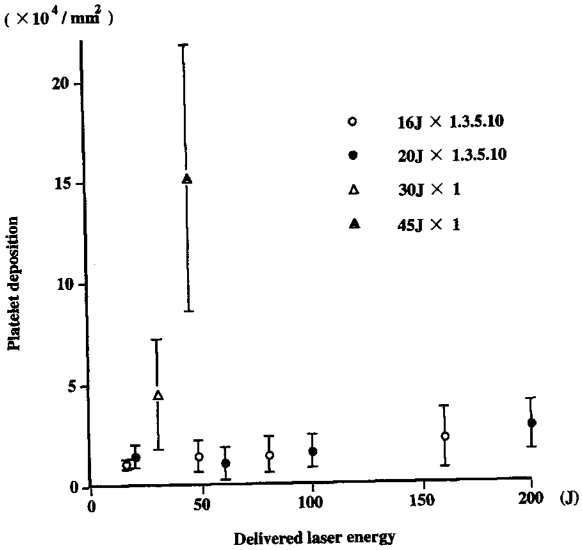

Fig.6. The relationship between the number of the platelet deposition and total laser energy in both single and repeated ablations.

RESULTS

Experiment I

Sixteen ablated lesions were made with the MTP, and all of them could be observed angioscopically (Fig. 3). No thrombus was recognized on all ablated lesions in case with a laser energy of 16 and 20 J. Small thrombus floating or sticking on the ablated area was observed in 1 of 4 cases (25%) with a laser energy of 24 J, and in 2 of 4 cases (50%) with that of 30 J.

Experiment II

A total of 84 lesions were ablated in 13 arteries. In the control group, the number of the platelet deposition was $0.42 \pm 0.15 \times 10^4$ /mm^2. In each single ablation of 16J, 20J, 24J, 30J, and 45J, the numbers of the platelet deposition were 1.05 ± 0.26, 1.47 ± 0.58, 1.05 ± 0.26, 4.54 ± 2.71, and $15.19 \pm 6.59 \times 10^4$ /mm^2 (Fig. 4). In cases with 30J and 45J, the numbers of platelet deposition were significantly higher than those in other groups. In 3, 5, and 10 times repeated ablations of 16J, the numbers of the platelet deposition were 1.46 ± 0.79, 1.49 ± 0.89, $2.30 \pm 1.51 \times 10^4$ /mm^2 (Fig. 5, above). In 3, 5, 10 times repeated ablations of 20J, the numbers of the platelet deposition were 1.07 ± 0.71, 1.62 ± 0.83, $2.80 \pm 1.19 \times 10^4$ /mm^2 (Fig. 5, below). The numbers of platelet deposition were gradually increased related to the frequency of laser ablations, and the numbers of platelet deposition of 10 repeated laser ablations of 16 J and 20 J were significantly higher than those of single ablation of 16 J and 20 J. The relationship between total energy and the numbers of platelet deposition were shown Fig. 6. In case with single ablation of 30 J and 45 J, the numbers of the platelet deposition were significantly higher than those in other groups.

DISCUSSION

Laser thermal angioplasty using the MTP was firstly reported in 1985 by Abela et al..[2] Following basic and experimental studies,[3,8,9,19] many clinical investigations of laser thermal angioplsty for arterial occlusive disease have been reported.[5,10,17,18,20] In these reports, fair results were noted not only in the short-term period but also in the long-term follow-up, and these excellent patency rates were considered to be brought by the anti-thrombogenecity of this intervention. Several experimental investigations concerning about platelet deposition after laser angioplasty were reported previously,[1,12,16] and laser ablation was considered to be less thrombogenic than balloon dilatation because the effects on the arterial wall were less traumatic.[1] Furthermore, an appropriate thermal effect to the arterial wall was considerd to prevent thrombogenesis. Borst et al. reported that platelet adhesion to the native or cultured human subendothelium was decreased after heating to 100℃.[6] They also showed that von-Willbrand factor was denaturated over the temperature of 70℃ in their experimental model[7] and concluded that laser thermal injury might result in denaturation and inactivation of subendotherial adhesive proteins that interact with blood platelets. However, it has not been yet cleared the relationship between delivered laser energy and the degree of platelet deposition which causes the thrombus formation in the acute stage and increase the proliferation of smooth muscle cells in the follow-up period. So, we experimentally investigated the thrombus formation after laser ablation angioscopically and evaluated the quantity of platelet deposition on the laser ablated area of various delivered energy. In this study, thrombogenesis and platelet deposition were evaluated 3 hours after laser angioplasty, because Wilentz et al. experimentally showed that marked platelet accumulation was observed at 30 minutes and remained active until 4 hours after balloon angioplasty.[22]

Angioscopic findings of this study revealed the fact that small thrombus floating or sticking on the laser ablated area sometimes existed when the delivered laser energy was above 24 J. From these findings, delivered energy for one laser ablation should be restricted within 24 J. Angioscopy reveals many findings which were not detected by conventional angiography.[4,11,21] It is able to distinguish atheromatous plaque and fresh or organized thrombus, observe the color of the lesion, and detect very fine thrombus or intimal flap. However, we sometimes overestimate the size of the lesion because the view of angioscopy is just like the view under magnifying glass. Furthermore, the sheath catheter was introduced from the distal side of the femoral arteries in this experimental study, and peripheral run off would be reduced by this catheter. The organization of the thrombus, red cell and white blood cell debris at the arterial wall may be accelerated under such an abnormal flow conditions.[15] So, we may also overestimate the frequency of thrombus formation after laser thermal angioplasty.

In our experimental study of measuring local platelet deposition, laser ablation was performed through the catheter from the carotid artery to avoid the reduction of the peripheral run off. This model was very similar to the clinical conditions in case of infrainguinal angioplasty. Some reports described that aggregating platelets released potent vasoactive substances and evoked vasospasm. Hehrlein et al. described that platelet deposition one hour after laser angioplasty was almost $30 \times 10^6/cm^2$ ($= \times 10^4/mm^2$), and extensive myointimal thickening was found eight weeks after laser ablation.[13] In their experimental studies, the temperature of the MTP rose up to 350℃ which was remarkably high energy compared with our conditions of laser ablation. Lam et al. reported that vasoconstriction occurred more severely when platelet deposition was in excess of $10 x 10^4/mm^2$ in the pig model.[14] In our experimental model, laser thermal ablation with a total energy of 45 J induced over $10 \times 10^4/mm^2$ platelet deposition. So, single ablation with an energy of 45 J was thought to be excess from the point of platelet deposition and vasoconstriction. From this study, we concluded that the optimal laser energy for one shot should be restricted within 24 J, and the repeated ablation was the most appropriate method of laser thermal ablation. Even in 10 times repeated ablation of 16-20 J, the number of platelet deposition was significantly lower than that of single ablation of 30-45J. Clinically we usually use the repeated ablation with the energy of 16-24 J to ablate the atheromatous lesion,[17,18] and this experimental data coincided with our clinical conditions from the aspect of thrombogenecity.

In order to decrease local platelet deposition, anticoagulation therapy is also one of the most important factor. In this experimental study, we used intravenous administration of heparin sulfate (100 IU/kg) only to prevent thrombosis caused by catheterization. In practice, anticoagulation therapy following laser thermal angioplasty may be necessary to prevent acute thrombosis and proliferating smooth muscle cells.

CONCLUSION

From the aspect of local platelet deposition, the optimal laser energy for one shot should be restricted within 24 J and the repeated ablation was the most appropriate method of laser thermal ablation.

REFERENCES

1. Abela, G.S., Tomaru, T., Mansour, M., Barbeau, G.R., Kaelin, L.D., Seeger, J.M., and Caravello, J.W. : J. Am. Coll. Cardiol. 1990. 15. 245A. Reduced platelet deposition with laser compared to balloon angioplasty.
2. Abela, G.S., Fenech, A., Crea, F., and Conti, R. : Lasers. Surg. Med. 1985. 5. 327 / 335. "Hot tip": Another method of laser vascular recanalization.
3. Abela, G.S., Crea, F., Seeger, J.M., Frazini, D., Fenech, A., Normann, S.J., Feldman, R.L., Pepine, C.J., and Conti C.R. : Am. J. Cardiol. 1985. 56. 983 / 988. The healing process in normal canine arteries and in atherosclerotic monkey arteries after transluminal laser irradiation.
4. Abela, G.S., Seeger, J.M., Barbieri, E., Franzini, D, Fenech, A., Pepine, C.J., and Conti, C.R. : J. Am. Coll. Cardiol. 1986. 8. 184 / 192. Laser angioplasty with angioscopic guidance in humans.
5. Barbeau, G.R., Seeger, J.M., Jablonski, S., Kaelin, L.D., Friedl, S.E., and Abela, G.S. : Clin. Cardiol. 1996. 19. 232 / 238. Peripheral artery recanalization in humans using balloon

and laser angioplasty.

6. Borst ,C., Bos, A.N., Zwaginga, J.J., Rienks, R., de Groot, P.G., and Sixma, J.J. : Cardiovasc. Res. 1990. 24. 665 / 668. Loss of blood platelet adhesion after heating native and cultured human subendothelium to 100^{o} celcius.
7. Bos, A.N., Post, M.J., de Groot, P.G., Sixma, J.J., and Borst, C. : Circulation.1993. 88. 1196 / 1204. Both increased and decreased platelet adhesion to thermally injured subendothlium is caused by denaturation of von Willebrand factor.
8. Consigny, P.M., Teitelbaum, G.P., Gardiner, G.A., and Kerns, W.D. : Cardiovasc. Intervent. Radiol. 1989. 12. 83 / 87. Effect of laser thermal angioplasty on arterial contractions and mechanics.
9. Consigny, P.M. and Gardiner, G.A. : J. Vasc. Intervent. Rad. 1991. 2. 253 / 260. Atherosclerotic rabbit iliac arteries: Comparison of balloon angioplasty and laser-assisted balloon angioplasty.
10. Diethrich, E.B., Timbadia, E., and Bahadir, I. : Eur. J. Vasc. Surg. 1989. 3. 61 / 70. Applications and limitations of laser-assisted angioplasty.
11. Grundfest, W.S., Litvack, F., Glick, D., Segalowitz, J., Treiman, R., Cohen, L., Foran, R., Levin, P., Cossman, D., and Carroll, R. : Circulation. 1988. 78. I-13 / I-17. Intraoperative decisions based on angioscopy in peripheral vascular surgery.
12. Hehrlein, C., Svenson, R.H., Mukusik, A.P., Chuang, C.H., Tuntelder, J.R., Tatsis, G.P., Littmann, L., and Thompson, M. : J. Cardiovasc. Surg. 1992. 33. 366 / 371. Thermal laser arterial injury and prostacyclin administration in dogs: Thrombotic and hyper plastic consequences.
13. Hehrlein, C., Chuang, C.H., Tuntelder, J.R., Tatsis, G.P., Littmann, L., and Svenson, R.H. : Circulation. 1991. 84. 884 / 890. Effects of vascular runoff on myointimal hyperplasia after mechanical balloon or thermal laser injury.
14. Lam, J.Y.T., Chesebro, J.H., Steele, P.M., Badimon, L., and Fuster, V. : Circulation. 1987. 75. 243 / 248. Is vasospasm related to platelet deposition? Relationship in a porcine preparation of arterial injury in vivo.
15. Merhi, Y., Guidoin, R., Provost, P., Leung, T.K., and Lam, Y.T. : Am. Heart. J. 1995. 129. 445 / 451. Increase of neutrophil adhesion and vasoconstriction with platelet deposition after deep arterial injury by angioplasty.
16. Mohan, S.R., Hawker, R.J., Wolinski, A.P., Dunham, J.A., Grimley, R.P., and Downing, R. : Angiology. 1992. 43. 11 / 21. Platelet accumulation after laser angioplasty-A scintigraphic assessment.
17. Okada, M., Yoshida, M., and Tsuji, Y. : Ann. Throac. Cardiovasc. Surg. 1995. 1. 157 / 162. Early and long-term results of laser angioplasty for the patients with occlusive arterial

disease.
18. Okada, M., Yoshida, M., and Tsuji, Y. : Angéiologie. 1990. 42. 121 / 127. Laser angioplasty for the peripheral and the coronary artery disease.
19. Oomen, A., van Erven, L., Vandenbroucke, W.V.A., Verdaasdonk, R.M., Slager, C.J., Thomsen, S.L., and Borst, C. : Lasers. Surg. Med. 1990. 10. 363 / 374. Early and late arterial healing response to catheter-induced laser, thermal, and mechanical wall damage in rabbit.
20. Sanborn, T.A., Cumberland, D.C., and Greenfield, A.J. : Radiology. 1988. 168. 121 / 125. Percutaneous laser thermal angioplasty : Initial results and 1-year follow up in 129 femoropopliteal lesions.
21. Tsuji, Y., Okada, M., Yoshida, M., and Nakamura, K. : J. Clin. Laser. Med. Surg. 1992. 10. 217 / 222. Clinical experience of laser angioplasty with agnioscopic guidance.
22. Wilentz, J.R., Sanborn, T.A., Haudenschild, C.C., Valeri, C.R., Ryan, T.J., and Faxon, D.P.: Circulation.1987. 75. 636 / 642. Platelet accumulation in experimental angioplasty: time course and relation to vascular injury.