TABLE 1. Basic Characteristics of the Patients of Macular Hole Surgery

Case No.	Eye No.	Eye	Age at Onset of MH (yrs)	Gender	MH Stage (Gass Classification)	Preoperative Latency (mos)	MH Size (DD)	Cataract Classification	Preoperative VA	Postoperative (Final) VA (Refraction)	Follow-up (mos)
1	1	Right	72	F	3	9	0.4	+2	20/125	20/100 (−2.50)	4
2	2	Left	57	M	3	2	0.2	+1	20/100	20/63 (−3.50)	5
3	3	Right	57	M	3	2	0.25	+1	20/63	20/20 (−4.50)	7
4	4	Left	55	F	3	1	0.25	+2	20/63	20/25 (−2.25)	7
5	5	Right	54	F	4	3	0.25	+1	20/200	20/63 (−0.75)	7
6	6	Right	69	F	2	4	0.4	+1	20/200	20/100 (−0.75)	6
7	7	Right	67	M	4	1	0.25	+1	20/200	20/32 (−1.25)	6
8	8	Left	78	M	2	3	0.2	+1	20/125	20/16 (−0.75)	4
9	9	Left	74	M	3	2	0.15	+1	20/63	20/16 (−1.00)	4
10	10	Left	60	F	3	1	0.33	+1	20/32	20/16 (−6.75)	5
	11	Right	60	F	3	2	0.33	+1	20/40	20/20 (−0.75)	5
11	12	Left	62	M	2	1	0.4	+2	20/400	20/50 (−0.35)	5
12	13	Left	55	M	3	0 (2 weeks)	0.5	+2	20/50	20/32 (−5.50)	5
13	14	Right	70	M	3	4	0.33	+1	20/200	20/100 (−0.60)	6
14	15	Right	78	M	2	1	0.4	+2	20/200	20/63 (−2.00)	6
15	16	Left	58	F	3	2	0.33	+0.5	20/50	20/25 (−1.25)	4

DD = disc diameter; F = female; M = male; MH = mascular hole; mos = months; VA = visual acuity; yrs = years.
Cataract grade +1, nuclear sclerosis; +2, moderate nuclear sclerosis; +3, advanced sclerosis.

10 eyes showed closed MH and 3 eyes showed unclosed MH. There was a bridge formation on the neural retina over a small amount of subretinal fluid (SRF) on the macular area in 7 eyes. Some of the bridges had a fistula-like structure (Figure 2). Posturing of patients having eyes with closed MH was stopped. On day 2, 2 of 3 eyes with unclosed MH documented by FD-OCT on day 1 were found to have a closed MH. Posturing of these patients then was stopped. From day 3 to day 7, the macular area could not be observed by FD-OCT because of strong light reflex from the lower surface of gas that occupied approximately 70% of the orbit. The volume of gas was reduced to less than 50% of the orbit after 7 days. Consequently, the macular area of all 16 eyes could be examined by FD-OCT, and MH closure was confirmed on day 9. The gas disappeared completely within 14 days in all eyes. At 1 month or later, the macular area could be observed clearly by both biomicroscopy and FD-OCT. Through the observations, no recurrence was found in any eyes.

Within 7 days, OCT-documented patterns of 12 eyes were determined; 3 eyes showed a simple closure pattern and 9 eyes showed a bridge formation pattern. At 1 month, 12 eyes showed a simple closure pattern and 4 eyes showed a bridge formation pattern (Table 2). Among 9 eyes having a bridge formation within 7 days, 6 eyes showed a simple closure pattern at 1 month. In contrast, no eyes with a simple closure pattern within 7 days changed to a bridge formation pattern at 1 month. BCVA improved in every eye. Comparatively worse postoperative BCVA (20/100) was found in those with long latency (4 months or longer). Four eyes with a bridge formation pattern at 1 month showed fair postoperative BCVA (20/63, 20/16, 20/20, and 20/25). There was no clear correlation between postoperative BCVA and any of the following: MH size, stage, and pattern of MH closure.

Two eyes showed transient intraocular pressure rises within 2 weeks after surgery, which were controlled by eye drops. There were no other defined adverse events in any eyes.

• **REPRESENTATIVE CASES:** *Case 10 (eye 11).* A 60-year-old woman noted visual disturbance and metamorphopsia for 2 months in her right eye and was referred to our hospital. Her BCVA was 20/40, and FD-OCT examination showed stage 3 MH of 0.33 disc diameter with parafoveal cystic change (Figure 2). She underwent a standard PPV with phacoemulsification and IOL implantation as described above. Posturing started immediately after surgery. At 3 hours, FD-OCT examination showed that the intraretinal cyst found before surgery was obscure and there was bridging of the neural retina without any MH structure, and the MH was thought to be closed as bridge formation type. On account of this result, the face-down posturing was stopped. The next day, the MH closure also was confirmed clearly by FD-OCT. The intraretinal cyst could not be observed. The fistula-like structure of the neural retinal bridge became evident on the SRF. At 1 month, BCVA improved to 20/20. FD-OCT examination showed a foveal depression with minimal SRF. A slit-like structure in the neural retinal bridge disappeared completely.

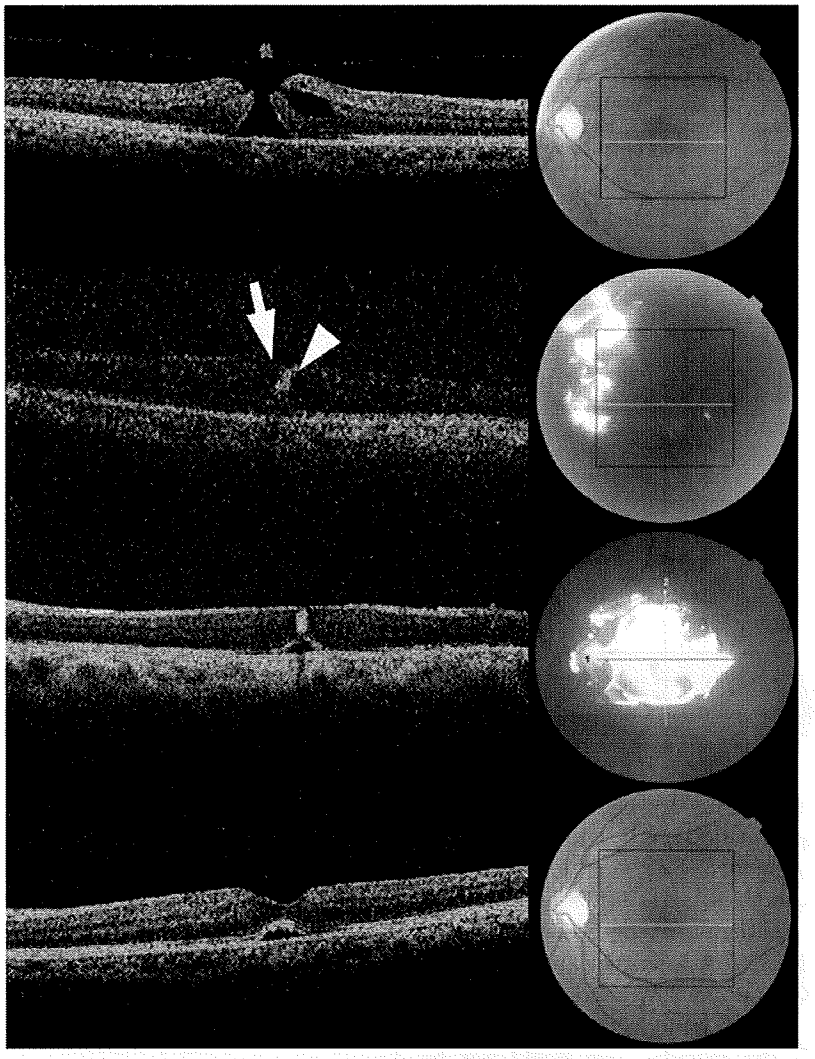

FIGURE 2. Fourier-domain OCT (FD-OCT) images and ocular fundus photographs after MH surgery obtained from Case 10 (eye 11). (Top row) Preoperative stage showing stage 3 MH. (Left) Several intraretinal cysts are found in the perifoveal retina. (Second row) Three hours after surgery. (Left) The inner surface of the retina appears to be continuous across the fovea (arrow). There is a hyperreflex spot on the macula (arrowhead). (Right) A strong light reflex is observed in ocular fundus. (Third row) One day after surgery. (Left) A bridging formation of tissue is found on the possible macular area. A hyperreflex spot also is visible in the retina like a fistula. Subretinal fluid beneath the fovea also is apparent. (Right) A strong light reflex also comes from the posterior retinal surface. (Bottom row) One month after surgery. (Left) MH is closed with foveal depression and subretinal space. (Right) MH disappeared. Square lines in the right figures show the area that was scanned serially by FD-OCT. Yellow or blue single lines also indicate the scanned sections shown on the left images.

Case 12 (eye 13). A 55-year-old male reported visual disturbance in his left eye for 2 weeks' duration. His BCVA was 20/50 at his first visit to our hospital. FD-OCT examination showed a stage 3 MH of 0.25 disc diameter (Figure 3). He also underwent a standard PPV with cataract surgery as described above. Posturing was started immediately after surgery. The next day, FD-OCT was performed and the hole appeared to remain open. At day 2, although the ocular fundus could be seen more clearly, the images by FD-OCT were unchanged. Therefore, posturing was continued for another 5 days until the macular area reappeared below the gas-bubble. During this period, the macular area could not be observed clearly by FD-OCT. FD-OCT at day 8 showed closure of the MH associated with foveal depression. BCVA improved to 20/32 at 5 months.

DISCUSSION

ALTHOUGH THE OCULAR FUNDUS IS OBSERVABLE EVEN in a gas-filled eye by conventional TD-OCT, a detailed examination of the macular area is hampered by a strong light reflex. Kasuga and associates reported that 4 (58%)

TABLE 2. Surgical Results and FD OCT Pattern after Macular Hole Surgeryat Each Time Point

	MH Closure Confirmed by FD-OCT								OCT Pattern	
Eye No.	Three Hours after Surgery	Day 1 (1 day after surgery)	% of Gas on Day 1	Day 2 (2 days after surgery)	Day 8 (8 days after surgery)	One Month after Surgery	Face-down Posturing Period (days)	Period (days) until Gas decreased to 50%	Within 7 days after Surgery	At One Month after Surgery
1	NA	Closed	100	NA	Closed	Closed	1	7	Simple	Simple
2	ND	Unclosed	90	Closed	Closed	Closed	2	6	Bridge	Bridge
3	ND	Unclosed	90	Closed	Closed	Closed	2	6	Bridge	Simple
4	NA	Closed	95	NA	Closed	Closed	1	8	Bridge	Simple
5	NA	Closed	96	NA	Closed	Closed	1	6	Bridge	Simple
6	NA	Closed	90	NA	Closed	Closed	1	6	Bridge	Simple
7	NA	Closed	90	NA	Closed	Closed	1	6	Bridge	Simple
8	NA	Closed	90	NA	Closed	Closed	1	5	Bridge	Bridge
9	NA	Closed	90	NA	Closed	Closed	1	7	Bridge	Simple
10	Closed	Closed	95	NA	Closed	Closed	1	8	Simple	Simple
11	Closed	Closed	100	NA	Closed	Closed	1	5	Bridge	Bridge
12	Closed	Closed	80	NA	Closed	Closed	1	5	Simple	Simple
13	NA	Unclosed	90	Unclosed	Closed	Closed	8	7	ND	Simple
14	NA	ND	ND	ND	Closed	Closed	6	7	ND	Simple
15	NA	ND	90	ND	ND	Closed	12	6	ND	Simple
16	NA	ND	100	ND	Closed	Closed	8	8	ND	Bridge

Bridge = bridge formation pattern; FD-OCT = Fourier-domain optical coherence tomography; Mh = macular hole; NA = not applicable (performed); ND = not done.

of 7 gas-filled eyes could be observed by TD-OCT on the day after surgery.[25] However, findings of the posterior retina depicted by TD-OCT would not necessarily prove MH closure because the macula is not identifiable in a gas-filled eye by several scans of TD-OCT. Especially in the immediate period after surgery, a foveal depression hardly exists in a gas-filled eye, which makes it more difficult to identify the macula correctly. In contrast, MH closure was confirmed reliably by FD-OCT, because it can cover a wider area by serially scanning every 40 μm. Indeed, 13 (81.3%) of 16 eyes could be examined clearly by FD-OCT during this period, which is much higher than that in Kasuga and associates' report (58%).[25] Without media opacity such as corneal haziness, it is likely that the more detailed postoperative morphologic features of the MHs can be examined by FD-OCT but not by conventional OCT immediately after surgery even in the eyes with gas tamponade. However, it is difficult to determine conclusively which is superior, because we did not perform a direct comparison using the same eyes in this study.

Using FD-OCT, the very early phase of the MH closure process after surgery was disclosed in detail. In the present series, a bridging formation of the neural retina was the first event after surgery. It seems that the neural retina was extended transversally, probably by the surface tension of the gas, release of tangential contraction by surgery, or both, resulting in so-called kissing or bridging of neural retina over the optically empty subretinal space. At day 1, these neural retinas were assumed to be just contacting each other, because gliosis or tissue repair was not likely to be completed within a day. However, as time advanced, the fistula-like structure became obscured and eventually disappeared. Possibly, as wound healing proceeded, the contacting retina fused with each other. At the same time, the subretinal space decreased over time (Figures 2 and 3). Interestingly, these processes are similar to those of spontaneous closure of the MH observed by OCT.[29,30] We believe that after the surface of the macula is sealed by tissue, it can keep contact using the negative pressure produced by fluid absorption in the RPE pump.[31] After this stage, no strong gas tamponade would be necessary and 1 day of face-down posturing may be sufficient.

Of important note is that the retinal cyst disappeared or was obscured before MH closure, which also was reported recently.[17] These findings support the hydration theory proposed by Tornambe.[31] We postulate that the critical factors for MH closure are relieving traction and isolating the hole from the vitreous fluid (posturing unnecessary), so that the intraretinal fluid can be pumped out by the RPE. This may be theoretical evidence that gas tamponade is not necessary for a long period.

Among 13 eyes that could be examined by FD-OCT within 2 days after surgery, 10 eyes showed signs of MH closure on day 1 (76.9%), 2 eyes on day 2 (15.4%), and 1 eye showed no sign of MH closure (7.7%). Although we did not have evidence, it is probable that 1 day of posturing would be sufficient for most eyes, but a prolonged tamponade would be necessary for some eyes to increase

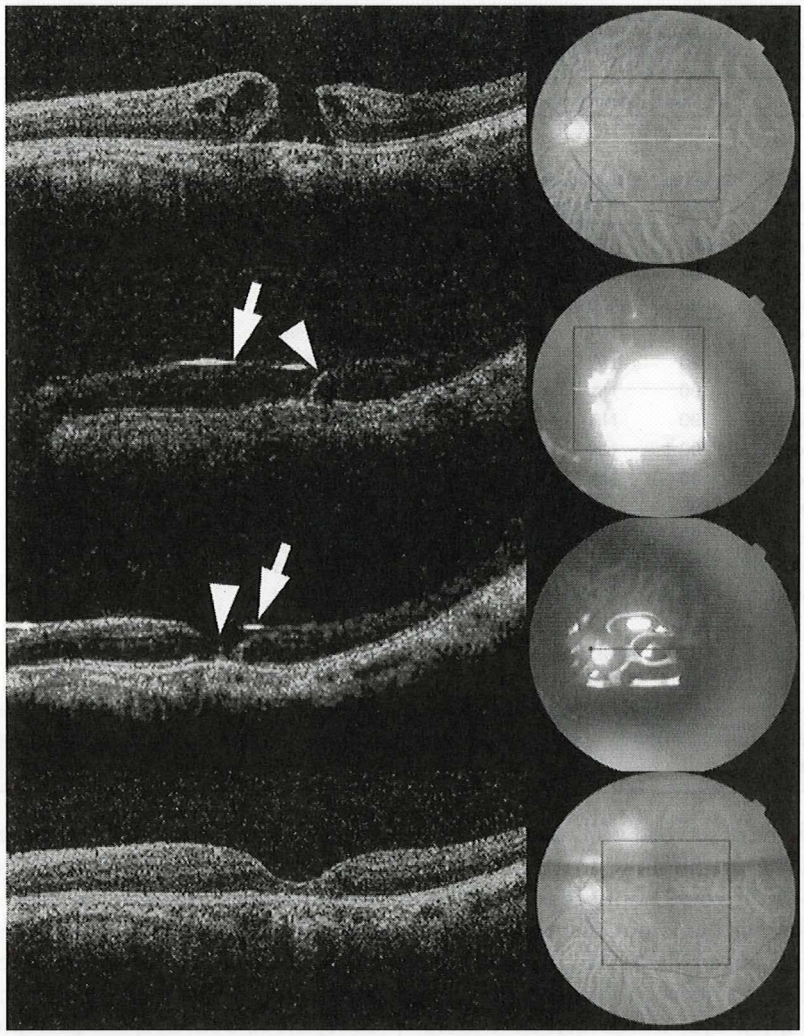

FIGURE 3. FD-OCT images and ocular fundus photographs after MH surgery obtained from Case 12 (eye 13). (Top row) Preoperative stage showing stage 3 MH. (Left) Several intraretinal cysts are found in the perifoveal retina. (Second row) One day after surgery. (Left) A fluid–gas interface is present (arrow). The neural retina at the temporal edge of the MH appears to be in contact with the retinal pigment epithelium, but there is a gap in the neural retina. The nasal edge of the MH is not well visualized in this image (arrowhead). (Right) A strong light reflex is noted in the ocular fundus. (Third row) Two days after surgery. (Left) Arrow indicates waterline. There is still a gap between the retina (arrowhead). (Right) A strong light reflex also comes from the posterior retinal surface. (Bottom row) 8 days after surgery. (Left) MH is closed with foveal depression. (Right) MH disappeared. An intraocular gas is still present. Square lines in the right figures show the area that was scanned serially by FD-OCT. Yellow or blue single lines also indicate the scanned sections shown on the left images.

the overall closure rate. To prove this, we would have had to stop posturing a patient with open MH and to demonstrate failure in such an eye, which would not be allowed ethically. Krohn reported that the success rate for 1 day of posturing was 87.5%, and for 1 week of posturing, it was 93.1%, although the results were statistically insignificant.[11] Hasler and Prünte performed surgery followed by 60% to 100% air tamponade with 2 days of posturing and observed that 90% of the MH was closed at day 2.[17] Their method of using a limited amount of air was superior because a rapid decrease of air allowed examination of the MH by OCT through the vitreous fluid from the early postoperative days. However, no additional tamponade is possible by continuing posturing, even if it is necessary. Hasler and Prünte performed the second surgeries for the eyes with unclosed MH in the early postoperative period. In contrast, in this study, 1 week of gas tamponade could close an MH that was found to be open at postoperative day 2 without any additional surgery (Case 12, eye 13). Phacovitrectomy with such a long-lasting gas as C_2F_6 is also a superior alternative method to avoid prolonged posturing,[16] but C_2F_6 gas remained for 4 to 6 weeks after surgery, and it would not be ideal for fast social rehabilitation.

There may be another concern with brief posturing. Takahashi and Kishi reported that a fragile or premature macula with a bridge formation was unstable and that

recurrence took place in such eyes.[19] Paques and associates reported that no evident cause of reopening was observed in most eyes with successfully closed MH and that reopening occurred after 8 months in that study; therefore, the length of posturing may not be an important factor for the recurrence of MH.[32] Although recurrence was not found in the present series, long-term follow-up is necessary.

Because all of the eyes in this study showed successful closure, the study provided no insight into whether posturing beyond 1 day was necessary for any patient. It is a theoretical possibility that we cannot answer from this study.

Our present method can be summarized as follows. Phacovitrectomy with ILM peeling is performed followed by full gas tamponade with 16% SF_6. Face-down posturing starts as immediately as possible after surgery. FD-OCT examination is performed from 3 hours to the day after surgery. When closure of the MH is confirmed, posturing is stopped. If not, posturing is continued until the macular area appears again below a gas-bubble (approximately 1 week). Using this method, most patients can be freed from needlessly prolonged posturing. A prolonged tamponade is carried out only for those who are found to have unclosed MHs within 2 days, which may not be saved by uniform short posturing.

We interpreted successful MH closure to have occurred after there was postoperative apposition of tissue across the fovea to bridge the MH. But, anatomic apposition may not necessarily equate with closure of MH. The bridge formation type of MH closure may represent incomplete closure where the central defect was too small to be imaged by FD-OCT rather than complete closure, because there is still SRF present. The simple closure pattern may well represent true MH closure, but could also just be apposition of the edges of the MH without a glial plug closing the hole. The intraocular gas-bubble remained in the eye, and it is likely that patients had some additional time when the gas-bubble contacted the MH because 16% SF_6 would last more than 1 week. Besides, other factors such as size and duration of the MH, duration of gas contact with the MH after surgery, or the use of ILM peeling may play a significant role. This should be remembered when interpreting the present results.

There are clear limitations in the present pilot study. Without question, the number of patients was small. The nonrandomized nature of patient selection carries a potential bias, and the sizes of MH were comparatively small. Some of these are inevitable with a prospective study. The present study was carried out during hospitalization, but there may be possible disadvantages for those with open holes or indeterminate imaging, because additional trips to the surgeon's office would be required as well as additional imaging studies in some cases. However, considering the noninvasiveness of the present method, the great merit of avoiding prolonged posturing, and the fact that no serious adverse event such as endophthalmitis was found, a tailor-made postoperative program based on early examination by FD-OCT after MH surgery should be justified. Although a further large-scale study is warranted, our present results emphasize the potential usefulness of FD-OCT in determining MH closure even at a very early postoperative stage with gas tamponade.

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Biosketch

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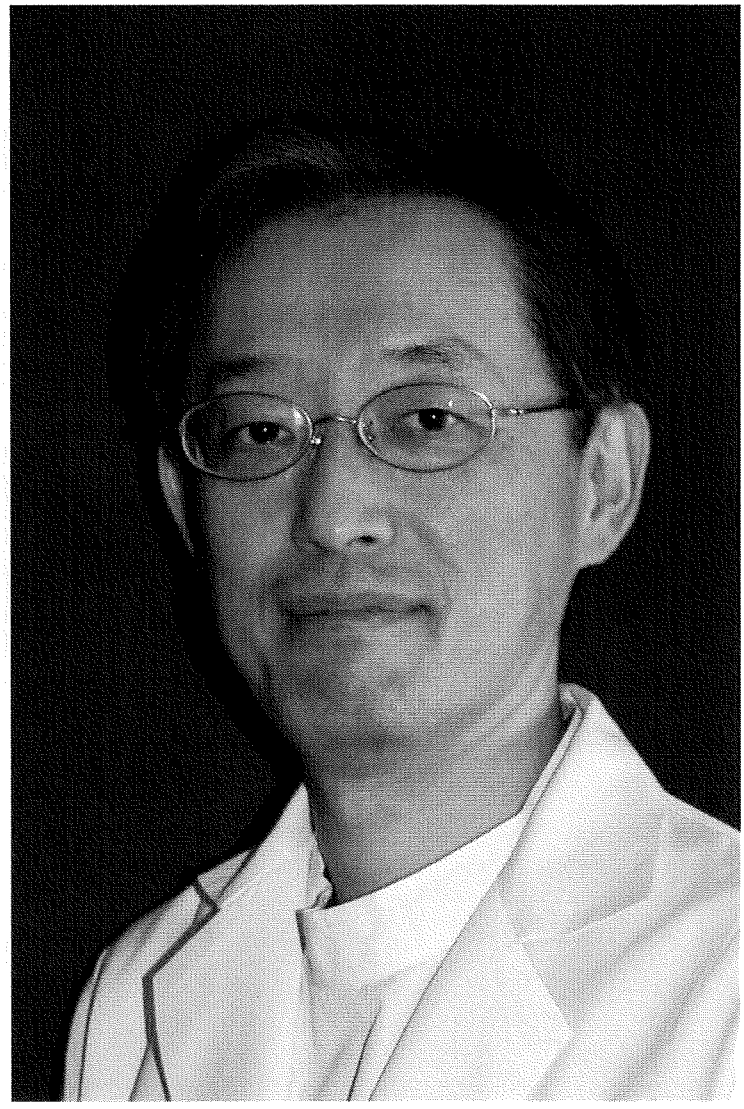

Biosketch

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CASE REPORT

Nasal and independent polypoidal lesions in polypoidal choroidal vasculopathy

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Abstract
Background Polypoidal vessels in polypoidal choroidal vasculopathy (PCV) are known to occur frequently in the macular and peripapillary regions. The aim of this study is to describe patients with polypoidal vessels that are nasal to the optic disc, being independent of macular polypoidal lesions.
Methods A 75-year-old man and a 65-year-old man with polypoidal vessels in the macula of both eyes were followed up through routine examinations including indocyanine green angiography and optical coherence tomography.
Results A polypoidal vessel located 1.5 disc diameters to the nasal margin of the disc was found in the right eye during the first examination in one case, and in the other case it developed during the follow-up period, after successful treatment using photodynamic therapy for the polypoidal lesion in the macula of the left eye. Indocyanine green angiography disclosed no continuity between the polypoidal vessels nasal to the disc and the polypoidal vessels in the macula region in either case. The nasal polypoidal vessels were not associated with exudative changes in either case, and the vessel in one case disappeared spontaneously without any treatment.
Conclusions The findings of this study demonstrate that PCV could involve regions outside the macula and occur in multiple areas independently. The results also indicate the dynamic nature and transitory appearance of polypoidal vessels. Polypoidal vessels nasal to the disc might be overlooked, especially in cases that are not associated with exudative changes, and careful examination might disclose more subclinical nasal polypoidal vessels. Further detailed examination would be helpful to gain a better understanding of the pathogenesis of PCV.

Competing interests The authors have no proprietary interest.

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Introduction

Polypoidal choroidal vasculopathy (PCV) has typical morphological features: dilated, aneurysmal, polyp-like structures (seen as reddish-orange structures) at the terminations of branching networks of large choroidal vessels, associated with recurrent serosanguineous detachments of the retinal pigment epithelium (RPE) and neurosensory retina and retinal exudation [1, 2]. The vascular abnormality appears to be in the inner choroid, and is composed of two basic elements on indocyanine green angiography (ICG-A): a polypoidal vessel that projects internally from the inner choroid toward the outer retina, and a branching vascular network. Polypoidal vessels have been reported to occur frequently in the macular and peripapillary areas [1, 2], and occasionally in the temporal peripheral fundus [3, 4]. We report on two patients with polypoidal vessels nasal to the optic disc and in the macula independently.

Report of cases

Case 1

A 75-year-old Japanese man presented with a 1-year history of decreased visual acuity in the left eye; the best corrected

visual acuity was 20/25 OD and 20/2000 OS. Examination of the left eye revealed several reddish-orange nodules suggesting polypoidal vessels, with serous retinal detachment and hard exudates in the macula. In the asymptomatic right eye, a focal elevated yellow lesion and a reddish-orange lesion (Fig. 1a, *arrows*) were observed inferior and superior to the centre of the fovea respectively. No associated exudative changes were seen, and the centre of the fovea revealed slight atrophic changes of the RPE. ICG-A, which was obtained using a fundus camera (TRC-50LX/IMAGnet, Topcon, Tokyo, Japan), revealed vascular networks that terminate in polypoidal vessels in the left eye, and polypoidal vessels inferior and superior to the centre of the fovea (Fig. 1b, *arrows*) in the right eye. PCV was diagnosed in both eyes. Funduscopic examination of the nasal quadrant in the right eye revealed a yellow nodule (Fig. 1a, *arrowhead*), which was shown as a focal elevation of the RPE by optical coherence tomography (Fig. 1a, *inset*), with slight atrophy of the RPE peripherally, 1.5 disc diameters to the nasal margin of the disc. The nodule was confirmed as a polypoidal vessel with ICG-A (Fig. 1b, *arrowhead*), having no continuity with the polypoidal vessels in the macula. The colour images and ICG-A were acquired by moving the camera head to the nasal side using a 50-degree lens. The patient was treated with two sessions of photodynamic therapy (PDT) using verteporfin (Visudyne, Novartis) in the left eye. The right eye was followed without any treatment, because the polypoidal lesion in the macula had not shown any significant changes and maintained good visual acuity (20/25) during the follow-up period of 31 months. Five months after the initial presentation, the nasal polypoidal vessel had disappeared when assessed by ICG-A (Fig. 1c), which was performed during a regularly scheduled follow-up visit to evaluate the necessity of retreating the left eye.

Case 2

A 65-year-old Japanese man, having decreased vision, with a prior history of laser photocoagulation for fundus hemorrhage 15 years before in the right eye was referred for evaluation. Best corrected visual acuity was 20/25 OD

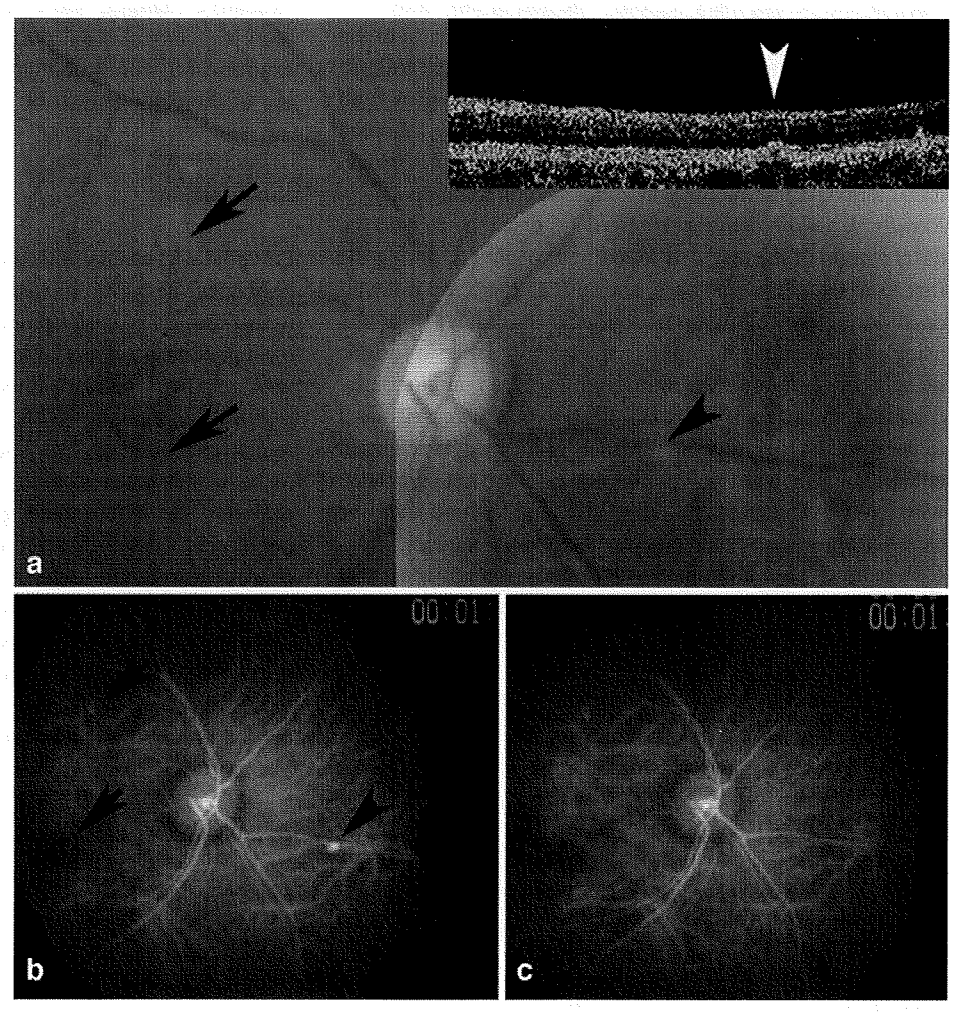

Fig. 1 **a** Fundus photograph of the right eye of case 1 at presentation showing focal elevated lesions (*arrows*) inferior and superior to the centre of the fovea, without exudative changes, and a yellow nodule (*arrowhead*) 1.5 disc diameters to the nasal margin of the disc, with slight atrophic change of the retinal pigment epithelium (RPE). *Inset*: optical coherence tomography (OCT) performed over the nasal lesion horizontally shows focal elevation of the RPE (*white arrowhead*). **b** Early phase of the indocyanine green angiography (ICG-A) at presentation reveals three polypoidal vessels (*arrows and arrowhead*) corresponding to the elevated lesions. The continuity between the polypoidal vessels in the macula and the polypoidal vessel nasal to the disc was not seen. **c** Early phase of the ICG-A taken 5 months after presentation showing the disappearance of the nasal polypoidal vessel

and 20/16 OS. Ophthalmoscopic examination of the right eye revealed a few reddish-orange nodules with hard exudates and serous retinal detachment in the macula; ICG-A revealed a few polypoidal vessels corresponding to the reddish-orange nodules. Ophthalmoscopic examination of the asymptomatic left eye revealed a few reddish-orange nodules inferior to the centre of the fovea, without any exudative changes such as detachment of the RPE and neurosensory retina, and hard exudates (Fig. 2a). ICG-A of the left eye showed polypoidal vessels corresponding to the reddish-orange nodules in the macula and choroidal hypofluorescence, within which a few dilated choroidal vessels were seen superior to the disc (Fig. 2b, *arrow*). The latter lesion showed slight atrophy of the RPE without elevation. At the first examination, no polypoidal lesions were observed in the nasal quadrants in either eyes. He was diagnosed as having PCV in both eyes. The right eye was treated with two sessions of PDT. Due to serous retinal

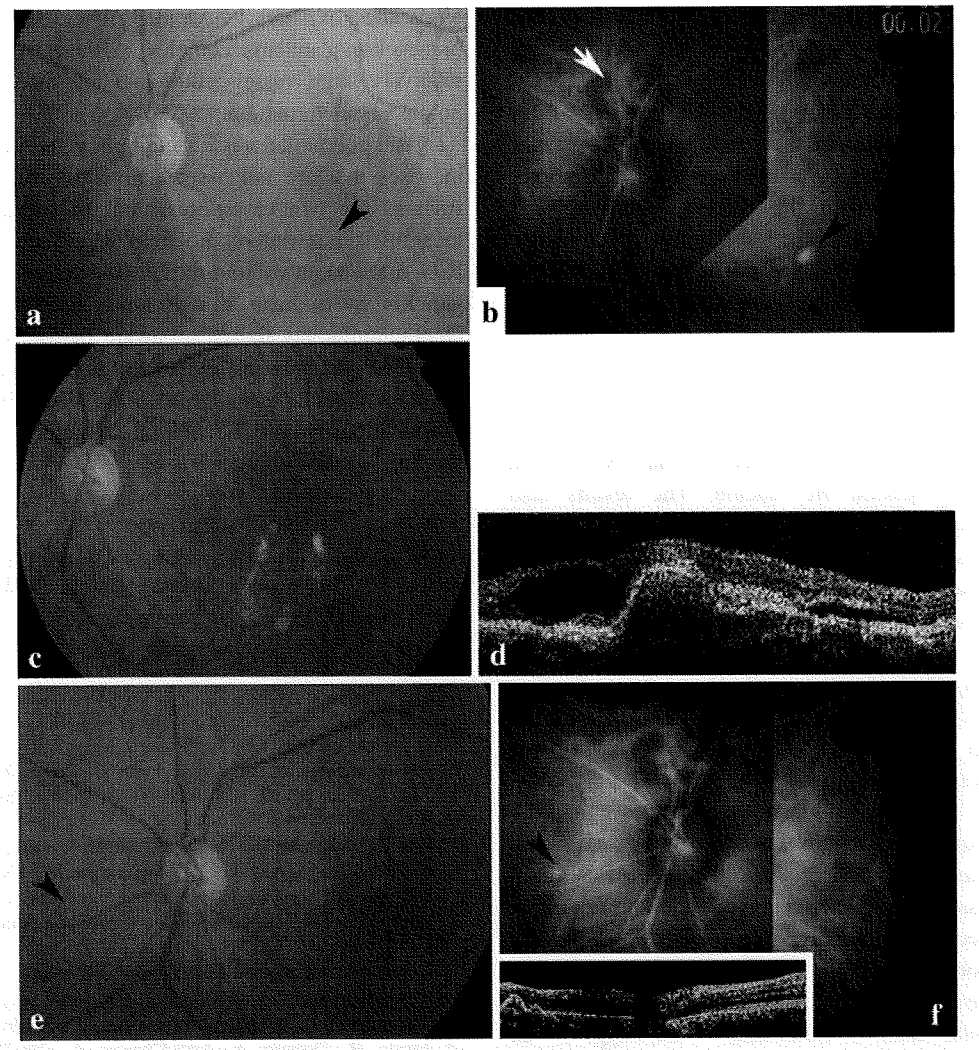

Fig. 2 **a** Fundus photograph of the left eye of case 2 at presentation, showing a few reddish-orange nodules inferior to the centre of the fovea (*arrowhead*) without exudative changes. **b** ICG-A at presentation reveals polypoidal vessels (*arrowhead*) corresponding to the reddish-orange nodules in the macula and area of choroidal hypofluorescence, within which a few dilated choroidal vessels (*arrow*) were seen superior to the disc. **c** Fundus photograph at 30 months after presentation and before the first photodynamic therapy (PDT), showing serous detachment of neurosensory retina, with hard exudates in addition to the reddish-orange nodules in the macula. **d** OCT performed over the macular lesion at 30 months after presentation reveals focal elevation of the RPE with serous retinal detachment and cystic macular edema. **e** Fundus photograph at 3 months after the second PDT showing disappearance of the reddish-orange lesion, serous retinal detachment and hard exudates. Of note is a new solitary small reddish-orange nodule (*arrowhead*) occurring 1.5 disc diameters from the nasal disc margin. **f** ICG-A taken 3 months after the second PDT, showing disappearance of polypoidal vessels in the macula and appearance of a polypoidal vessel (*arrowhead*) corresponding to the new reddish-orange nodule. The continuity between the nasal polypoidal vessel and the original polypoidal lesion in the macula was not shown. *Inset*: OCT performed over the nasal polypoidal vessel horizontally showing focal elevation of the RPE

detachment and hard exudates that developed in the left eye, and a decreased visual acuity of 20/40 (Fig. 2c,d), the left eye was also treated with PDT to cover the polypoidal vessels in the macula 30 and 34 months after initial presentation. According to the standard protocol, fluorescein green angiograms were evaluated to determine the greatest linear dimension (GLD), and a 689-nm diode laser was applied for 83 seconds with a light dose of 50 J/cm^2. The GLD at each time was 4100μm and 3700μm respectively. Three months after the second PDT, the polypoidal vessels in the macula and serous retinal detachment disappeared, but a new solitary small polypoidal vessel occurred 1.5 disc diameters from the nasal optic disc margin (Fig. 2e,f). The continuity between the nasal polypoidal vessel and the original polypoidal lesion in the macula was not shown. The nasal polypoidal vessel was not associated with serous/hemorrhagic detachment of the RPE and neurosensory retina, nor hard exudates until the last follow-up examination (23 months after the appearance of the nasal polypoidal vessel). Visual acuity remained stable at 20/32.

Discussion

The findings of this study demonstrate that PCV could involve regions outside the macula. The results also indicate the dynamic nature and transitory appearance of polypoidal vessles.

The patients presented here had typical polypoidal lesions in the macula of both eyes. Polypoidal vessels were also observed 1.5 disc diameters to the nasal margin of the disc. The nasal polypoidal vessel was found during the first examination of case 1, while in case 2 it developed during the follow-up period after successful treatment of the polypoidal vessels in the macula. The nasal polypoidal vessel in case 1 disappeared without any treatment, and the vessels in both cases were not associated with any exudative changes. It is difficult to conclude that nasal polypoidal vessels are less active than polypoidal vessels in the macula, because some polypoidal vessels in the macula were also reported to disappear spontaneously.

Notably, the nasal polypoidal vessel showed no continuity with the macular lesion in either case, which suggests that polypoidal vessels could occur in multiple areas independently. Regarding the location of pathologic vessels, choroidal neovascularization in typical exudative age-related macular degeneration (AMD) is known to occur in the macula mainly, while polypoidal vessel formation in PCV occurs frequently in the macular and the peripapillary regions. Recently, genetic differences in the elastin gene between patients with neovascular AMD and patients with PCV have been reported [5], supporting previously reported different histopathologic aspects of the two diseases in Bruch's membrane and in the walls of pathologic vessels containing elastic layers. In clinicopathologic studies of patients with PCV, altered RPE–Bruch's membrane–choriocapillaris complex and underlying arterioles with marked sclerotic changes and dilated venules were observed [6–8]. In addition, cavernous vascular channels that originated from branches of the short posterior ciliary arteries (PCAs) were documented in another clinicopathologic report [9]. Indeed, the location of frequently occurring polypoidal vessels in the macular and peripapillary areas, and even the nasal area to the disc, would overlap the entry sites of short PCAs [10]. The RPE–Bruch's membrane–choriocapillaris complex in those areas where such relatively large vessels enter may be susceptible to polypoidal vessel formation.

Nasal polypoidal vessels might be overlooked, especially in cases that are not associated with exudative changes, such as the two cases presented. In addition, this nasal location is just out of the field when colour images and ICG-A are taken without moving the camera head or asking patients to look in a particular direction consciously. Ophthalmologists should be aware that polypoidal vessels can occur not only in the macular and peripapillary region but also in the nasal region to the disc. Further detailed examinations would be helpful to gain a greater understanding of the pathogenesis of PCV.

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● *Original Contribution*

SONOTHROMBOLYSIS FOR INTRAOCULAR FIBRIN FORMATION IN AN ANIMAL MODEL

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Abstract—Vascular diseases such as diabetic retinopathy or retinal arterial occlusion are always associated with retinal and/or choroidal vasculopathy and intravascular thrombosis is commonly found. The ultrasound (US) therapy is a recently developed technique to accelerate fibrinolysis and it is being applied to some clinical fields. The present study was to observe the effects of extraocular US exposure on intraocular fibrin, which is a deteriorating factor in various ocular diseases. Tubes containing human blood (2 mL) in the following groups were irradiated with US; US alone, US with tissue plasminogen activator (tPA), tPA alone, and saline (control). Fibrinolysis was quantified by measuring D-dimer after 2 h. In rat eyes, intracameral fibrin (fibrin formation in the anterior chamber of the eye) was induced by YAG-laser-induced iris bleeding. Then, eyes in the following groups were irradiated with US; US alone, subconjunctival tPA alone, US and subconjunctival tPA, control. Intracameral fibrin was scored on day 3 (3+ maximum to 0). The temperatures of rat eyes were measured by infrared thermography. Histologic evaluation was also performed. D-dimer was increased by US with statistical significance ($p < 0.05$) or tPA ($p < 0.01$). D-dimer in US with tPA group was significantly higher than either US alone or tPA alone group ($p < 0.01$). In rat eyes, the average intracameral fibrin score on day 3 was 1.4 in control group and 1.2 in subconjunctival tPA alone group; however, it decreased significantly in the US alone group (0.75; $p < 0.05$, vs. control), US and subconjunctival tPA group (0.71; $p < 0.01$, vs. control). The temperature was less than 34 °C after US exposure. No histologic damage was observed. US irradiation from outside accelerated intracameral fibrinolysis without causing apparent tissue damage. This noninvasive method might have therapeutic value for intraocular fibrin. (E-mail: tsakamot@m3.kufm.kagoshima-u.ac.jp) **© 2009 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Ultrasound, Fibrinolysis, Thrombolysis, Sonothrombolysis, Tissue plasminogen activator.**

INTRODUCTION

Vascular diseases such as diabetic retinopathy are the leading causes of legal blindness in adults in western societies. These diseases are always associated with retinal and/or choroidal vasculopathy and intravascular thrombosis is commonly found to some extent (Vine and Samama 1993). Among them, retinal arterial occlusion (RAO) has a poor prognosis (Hayreh 2008). Furthermore, fibrin formation in the vitreous and/or anterior chamber is sometimes observed after intraocular surgery, leading to secondary tissue damage (Akassoglou et al. 2004; Jaffe et al. 1990; McDonald et al. 1990; Toth et al. 1991). To remove thrombus or fibrin, a fibrinolytic agent such as tissue-plasminogen activator (tPA) is sometimes used (Feltgen et al. 2006; Hayreh 2008; Kattah et al. 2002; Noble et al. 2008; Richard et al. 1999; Weber et al. 1998). However, the effect of fibrinolytic agents on these diseases is controversial and the potential toxicity of high doses of tPA cannot be neglected (Yamamoto et al. 2008; Yoeruek et al. 2008). Surgical removal of intravascular thrombus is another approach but its therapeutic value is also controversial (García-Arumí et al. 2006; Opremcak et al. 2008). Above all, the invasiveness and the potential risks of surgical embolus removal would make it difficult for physicians to accept these treatments.

The use of ultrasound (US) to accelerate fibrinolysis for thrombolytic therapy is a developed technique. It was

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evaluated by previous studies and has been found to have effects but less invasive potential for fibrinolysis (Francis et al. 1992; Holland et al. 2008; Hong et al. 1990; Lauer et al. 1992; Tachibana 1992; Tachibana K and Tachibana S 1995, 1997; Trübestein et al. 1976). Therefore, it is being applied in some clinical fields including peripheral vascular occlusions, acute myocardial infarctions (Cohen et al. 2003), occluded arteriovenous dialysis grafts (Pfaffenberger et al. 2005) and acute ischemic stroke. Early investigators showed that transcranial US increases fibrinolytic activity (Francis et al. 1995). Although a translational study using large US probes was hampered by an increased rate of intracerebral hemorrhage, phase-II Combined Lysis of Thrombus in Brain Ischemia Using Transcranial Ultrasound and Systemic t-PA trial that randomly assigned 126 patients with middle cerebral artery occlusion showed favorable results (Alexandrov et al. 2004; Daffertshofer et al. 2005).

In comparison to intracranial or visceral lesions, intraocular lesion is expected to be suitable for this treatment because responses to the treatment can be monitored in detail by direct observation and the therapeutic efficacy might be achievable with less power. So far, to the best of our knowledge, there have been no reports on sonothrombolytic treatment for ocular fibrin formation. In this study, we report on this new promising treatment approach for intracameral fibrin (fibrin formation in the anterior chamber of the eye) and evaluate its effects.

MATERIALS AND METHODS

In vitro study

Fibrin clot preparation. Venous blood was withdrawn from four healthy volunteers after they provided informed consent. Whole blood (2 mL) was immediately placed in disposable culture tubes. The tubes containing the blood were then placed in a 37°C water bath for 2 h referring to the previously described method (Frenkel et al. 2006).

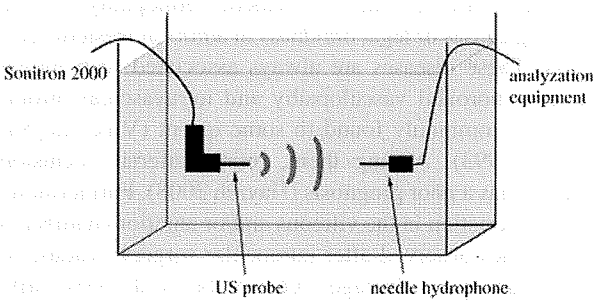
US treatment. US was irradiated using a commercially available machine (Sonitron 2000; Richmar, Inola, OK, USA) with a 6 mm unfocused probe directly to the blood in the tubes (Borex, 12 × 75 mm disposable culture tubes, composed of borosilicate glass) in a tank of water at 37°C for various time intervals. Based upon our preliminary experiment, the parameters of US were determined as follows: frequency of 1.0 MHz, duty cycle of 5.2%, pulse repetition frequency of 20 Hz and the derated spatial-peak pulse-average intensity ($I_{SPPA.3}$) of 10.123 W/cm^2. First, US was irradiated with different exposure times, 5 min or 20 min (n=8, each). In addition, the temperature in the tube was measured after US irradiation for 20 min under the same condition (n = 4). Next, the experimental groups were divided into the following: US exposure alone (US, n = 8), US exposure with tPA (US + tPA, n = 8), tPA alone (tPA, n = 8) and saline alone (no US, no tPA, control, n = 8). tPA (Cleactor; Eisai, Tokyo, Japan) was diluted with saline (40 IU/μL) just before the experiment. Before the clots were exposed to US, tPA and saline (total 500 μL) were added to the tubes and incubated in a 37°C water bath for 2 h.

D-dimer assays. Because D-dimer is an indicator of fibrin-degraded products, a commercial D-dimer assay kit (Diagnostica Stago, Parsippany, NJ, USA) was used to measure D-dimer levels 2 h after treatment. The assays were performed according to directions provided by the manufacturer.

In vivo study

Safety of US exposure. Before application of US with animals, the output level of US was evaluated. We examined the safety of Sonitron 2000 with a 3 and 6 mm unfocused probes in consideration of application to human eyes. In brief, we measured acoustic power using a needle hydrophone in a water tank and assessed and computed some acoustic parameters (Schema 1). US was irradiated under the following conditions: frequency of 1.0 MHz, high or low intensity mode indicated on the device and duty cycle of 5.2% or 100%. Safety conditions referred to information of manufacturers seeking marketing clearance for diagnostic ultrasound systems and transducers by the Food and Drug Administration of the United States Department of Health and Human Services (FDA) (http://www.fda.gov/cdrh/ode/guidance/560.html#2).

Animals. All animals were used humanely in accordance with the approval of our institutional animal care committee and the ARVO statement on the Use of Animals in Ophthalmic and Vision Research. Brown-Norway rats (male; age 8-weeks old, 250 g) were purchased from KBT Oriental Co., Ltd. (Fukuoka, Japan).



Schema 1. The simplified schema of the measurement system to evaluate the output level of US. Measurement system using a needle hydrophone in a water tank to assess and compute some acoustic parameters of Sonitron 2000 with a 3 and 6 mm unfocused probes.

Induction of intracameral fibrin clot formation and evaluation. In each of the following procedures, Brown-Norway rats were anesthetized with an intramuscular injection of ketamine hydrochloride (50 mg/kg) and xylazine hydrochloride (20 mg/kg), and the ocular surface was anesthetized with a topical instillation of 0.2% oxybuprocaine hydrochloride eye drops (Santen Pharmaceutical Co., Ltd., Osaka, Japan). Intracameral bleeding was induced by a neodymium-doped yttrium aluminium garnet (Nd:YAG) laser according to previous report (Sakamoto et al. 1999). Nd:YAG laser radiation on the iris is a standard treatment in clinical ophthalmology for glaucoma and appears to be a safe (Drake 1987; Tomey et al. 1987). In this study, laser radiation was performed by the Q-switched Nd:YAG laser system mounted on a slit lamp (Ellex Japan Inc., Osaka, Japan) using energy setting of 1.2 mJ per shot and single pulse mode. Nd:YAG laser were shot at six points on the iris (0, 2, 4, 6, 8 and 10 o'clock) with the spot size of 8 μm. In order to confirm the selectivity of YAG laser treatment, three eyes were enucleated immediately and fixed with 3.7% formaldehyde in phosphate buffered saline (PBS), dehydrated with a graded alcohol series and embedded in paraffin. The sections were cut and stained with hematoxylin and eosin. All of the specimens were then observed by two masked observers who received no information about the specimens. After laser shots, moderate to severe bleeding occurred immediately, clot and fibrin was discerned on the surface of the iris and lens on the next day. The value of clot formation in the anterior chamber was graded using surgical microscopy according to the previously described method (Sakamoto et al. 1999). Briefly, the criteria were defined as follows: 3+, clot or bleeding occupies more than one-third of the anterior chamber; 2+, between one-fifth and one-third of anterior chamber; 1+, less than one-fifth of anterior chamber; 0, no clot or bleeding. The eyes were observed by surgical microscopy everyday and photographs were taken on the next day (day 1) and fourth day (day 3) by masked observers.

Sonothrombolysis. The day after the laser shots, a 3 mm US probe was placed directly onto the corneal surface, coupling with a gel, hydroxyethyl cellulose (Senju Pharmaceutical Co. Ltd., Osaka, Japan) of the rats under general anesthesia and US was irradiated. The parameters of US were set at frequency of 1.0 MHz, duty cycle of 5.2%, pulse repetition frequency of 20 Hz and $I_{SPPA.3}$ of 0.228 W/cm^2 and US was irradiated for 5 min and the experimental groups were divided as follows: US alone (US group, $n = 14$), US and subconjunctival tPA (US and subconjunctival tPA group, $n = 14$), subconjunctival tPA alone (tPA group, $n = 14$) and no treatment (control, $n = 14$). When t-PA was applied, 50 μL of t-PA (2000 IU) after diluting with saline (final concentration 40 IU/μL) was injected into the center of the tarsal conjunctiva using a syringe with a 30-gauge needle under surgical microscopy. Given that tPA weights 68 kDa, subconjunctival injection of tPA would have a limited effect, however, the thrombus was dissolved even a little intracameral administration in our preliminary study. So, this method was done as a topical administration.

Ocular surface temperature and histologic findings. Brown-Norway rats were first anesthetized as described above. A 3 mm US probe was placed directly onto the corneal surface and US was irradiated under the following conditions: frequency of 1.0 MHz, high intensity mode (indicated on the device) and duty cycle of 5.2% or 100%. The temperature was monitored for 5 min using infrared thermography (TH6200R, NEC Co., Ltd., Tokyo, Japan). For the microscopic analysis, the eyes were enucleated after 48 h and were examined histologically the same way as above. More than six eyes of each group were examined.

Statistical analysis. All values were expressed as mean ± SD. Analysis of variance with paired *t*-test was used to determine the significance of the difference in a multiple comparison. Differences with a p value of less than 0.05 were considered to be significant.

RESULTS

In vitro study

US exposure time. US exposure was examined under the above conditions for 5 min or 20 min (exposure time). The results showed that D-dimer was significantly increased by US exposure and that 20 min exposure of US resulted in significantly higher levels than 5 min exposure (Fig. 1, $p < 0.01$). In this condition, there was no

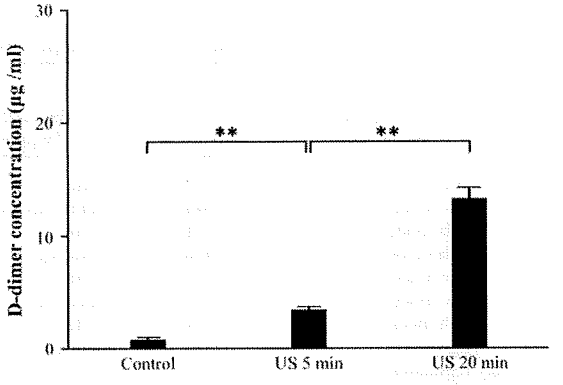


Fig. 1. Amount of D-dimer after US exposure. D-dimer increased after US exposure in a time-dependent fashion (paired *t*-test, $^{**}p < 0.01$). Control; neither US nor tPA.

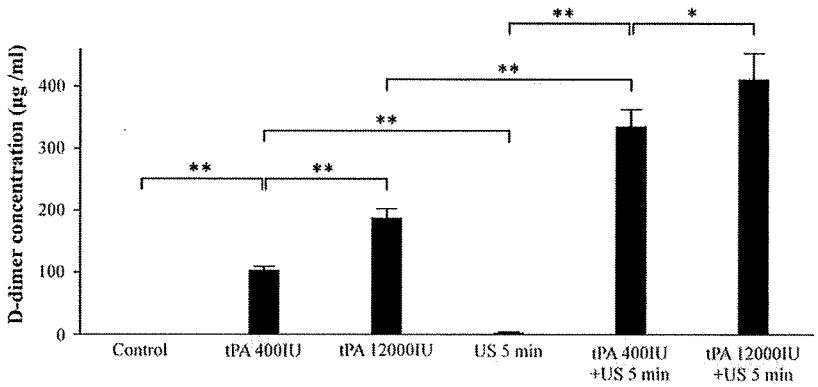


Fig. 2. Amount of D-dimer after US exposure and tPA. D-dimer was increased by tPA in a dose dependent fashion. Simultaneous US exposure and tPA significantly increased the amount D-dimer (paired *t*-test, $*p < 0.05$, $**p < 0.01$).

change in temperature from 37°C after US exposure in all experiments.

Effects of US and tPA. D-dimer was significantly increased by tPA application and also showed a dose-dependent effect (Fig. 2, $p < 0.01$). Because 5 min exposure was sufficient to obtain treatment effects, the following US exposure times were fixed for 5 min. As a result, D-dimer was increased by tPA in a dose-dependent fashion. Simultaneous US exposure and tPA significantly increased D-dimer (Fig. 2).

Safety of US exposure. With a 6 mm probe, $I_{SPPA.3}$ was less than 28 W/cm^2 but the derated spatial-peak temporal-average intensity ($I_{SPTA.3}$) was far more than 17 mW/cm^2 in all conditions. With a 3 mm probe, $I_{SPPA.3}$ was less than 28 W/cm^2 and $I_{SPTA.3}$ was also less than 17 mW/cm^2 under the condition of a duty cycle of 5.2%. All computed acoustic parameters were referred in Table 1.

In vivo study

Sonothrombolysis. Before US irradiation, the fibrin score was approximately 2.4 and there were no statistical differences between groups. In the controls, intracameral fibrin clot decreased gradually over time, and the average score on day 3 was 1.4 ± 0.21. While eyes that received subconjunctival tPA injection alone showed a slight decrease of clots, and the average score was 1.2 ± 0.19. There was no statistically significant difference with controls. In contrast, eyes that received US alone or both subconjunctival tPA and US showed apparent decreases of clots and the average scores decreased to 0.75 ± 0.13 and 0.71 ± 0.11, respectively (control vs. US alone; $p < 0.05$ and control vs. US with subconjunctival tPA; $p < 0.01$) (Figs. 3 and 4). During the experimental course, no pathologic change such as edema or new bleeding was observed in any of cornea, anterior chamber, iris or lens by surgical microscopic observation (Fig. 4).

Ocular surface temperature. Before US exposure, the surface temperature was approximately 25°C (less than 32 °C in the periocular area) (Fig. 5A). Immediately after US exposure, the temperature started to increase. Under the US condition: frequency of 1.0 MHz, duty cycle of 5.2%, pulse repetition frequency of 20 Hz and $I_{SPPA.3}$ of 0.228 W/cm^2, the temperature increased slightly (approximately 28.5°C) and always remained less than about 32°C in the periocular area (Fig. 5B). However,

Table 1. Acoustic output level of ultrasounds about Sonitron 2000

Probe	Intensity (Indicated on the device)	Duty cycle	Pulse repetition frequency	Peak rarefactional acoustic pressure (MPa)	$I_{SPPA.3}$ (W/cm^2)	$I_{SPTA.3}$ (mW/cm^2)
6 mm	Low mode	5.2%	20 Hz	0.397	5.409	280.169
6 mm	Low mode	100%	continuous wave	0.425	5.982	5933.022
6 mm	High mode	5.2%	20 Hz	0.545	10.123	524.369
6 mm	High mode	100%	continuous wave	0.575	11.220	11128.540
3 mm	Low mode	5.2%	20 Hz	0.070	0.177	9.190
3 mm	Low mode	100%	continuous wave	0.065	0.155	154.032
3 mm	High mode	5.2%	20 Hz	0.083	0.228	11.791
3 mm	High mode	100%	continuous wave	0.062	0.156	154.484
FDA safety regulation					<28	<17

The frequency of this machine was fixed 1.0 MHz. $I_{SPPA.3}$ was less than 28 W/cm^2 under all conditions. Meanwhile $I_{SPTA.3}$ was less than 17 mW/cm^2 under only the condition of a duty cycle of 5.2% with 3 mm probe.

$I_{SPPA.3}$ = a derated spatial-peak pulse-average intensity; $I_{SPTA.3}$ = a derated spatial-peak temporal-average intensity.

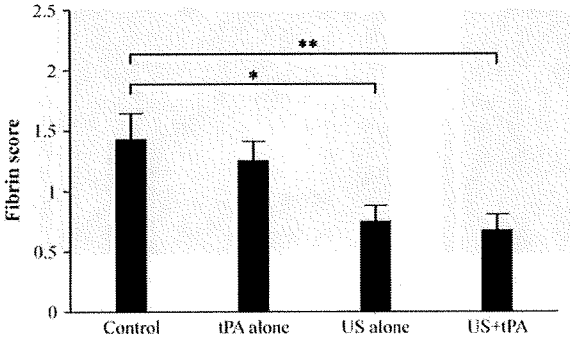


Fig. 3. Intracameral fibrin score on day 3. Intracameral fibrin clot decreased gradually over time and the average score on day 3. While the eyes that received subconjunctival tPA injection alone showed mild decrease of clots. There was no statistically significant difference from controls. In contrast, the eyes that received US alone or both tPA and US showed apparent decrease of clots (paired *t*-test, $*p < 0.05$, $**p < 0.01$).

under the US condition: frequency of 1.0 MHz, duty cycle of 100%, continuous wave mode and $I_{SPPA.3}$ of 0.156 W/cm^2, the temperature increased considerably (to about 34°C) more than the periocular area (Fig. 5C).

Histological findings. In immediate histological finding after YAG laser treatment, there was not any apparent damage in other ocular tissues including cornea and retina (Fig. 6). After US irradiation, the structure of the cornea was well preserved and neither inflammatory cell infiltration nor stromal edema was found. Retinal structure was also well preserved and neither inflammatory infiltrate nor hemorrhage was observed either (Fig. 7).

DISCUSSION

In this study, we found that US exposure significantly accelerated the disappearance of intracameral fibrin without causing any apparent damage. It is further important that this effect was accomplished within the range of safety condition.

Many reports show that US exposure accelerates fibrinolysis in vitro and combining US with various thrombolytic agents, *e.g.*, heparin sulfate, aspirin, urokinase type-plasminogen activator or tPA further accelerated fibrinolysis (Francis et al. 1992; Holland et al. 2008; Hong et al. 1990; Lauer et al. 1992; Tachibana 1992; Trübestein et al. 1976). In the present study, US exposure significantly enhanced fibrinolysis *in vitro* without thermal elevation; this effect was augmented by tPA. Although we cannot know the exact mechanism by which US accelerated fibrinolysis, most currently accepted or possible explanations are that US exposure changes the structure of clots and alters the drug distribution, resulting in deeper penetration into the clots by US, namely due to acoustic cavitations, bubble vibration, and their collapse (Datta et al. 2006; Francis et al. 1992; Hong et al. 1990; Lauer et al. 1992; Tachibana 1992; Trübestein et al. 1976). Of note, we have to interpret the results cautiously. In this study, we used borosilicate glass

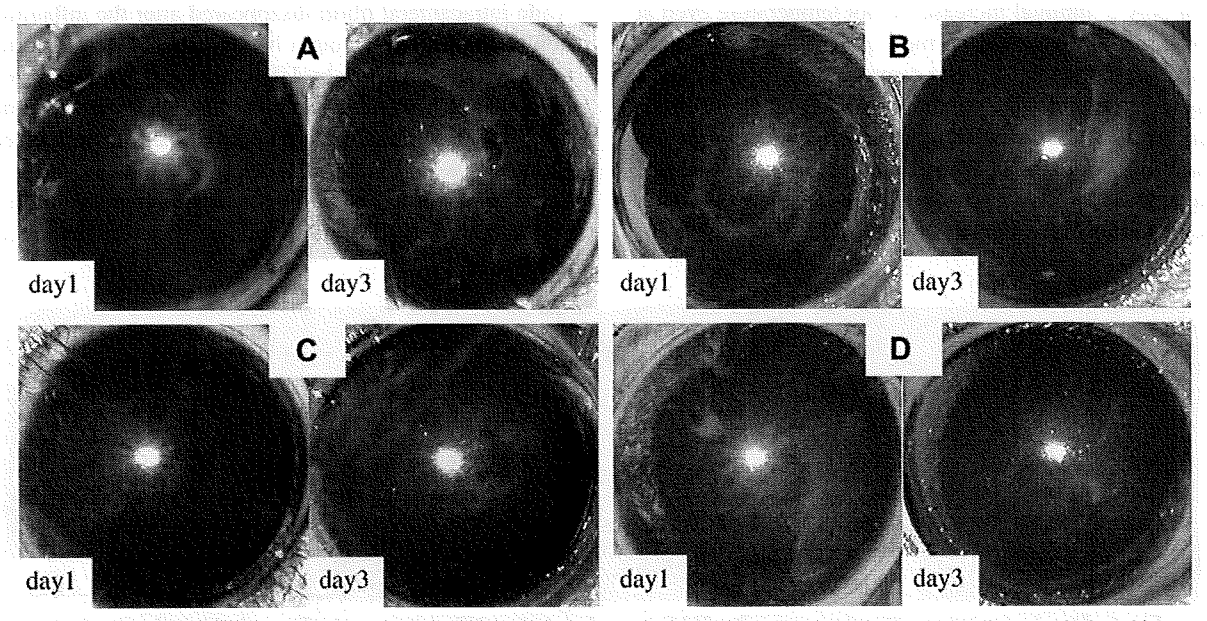


Fig. 4. Representative photograph of rat eyes after bleeding followed by treatment. Intracameral bleeding was induced by Nd:YAG laser shot on iris followed by subconjunctiva tPA injection and/or US exposure. Intracameral fibrin was scored on day 3. (A) Control. Fibrin clots decreased gradually. (B) US exposure alone. Fibrin clots was apparently decreased. (C) Subconjunctival tPA injection alone. Fibrin clots decreased gradually and there was no apparent difference from controls. (D) Subconjunctival tPA plus US exposure. Fibrin clots was significantly decreased and no clot was observed on day 3.

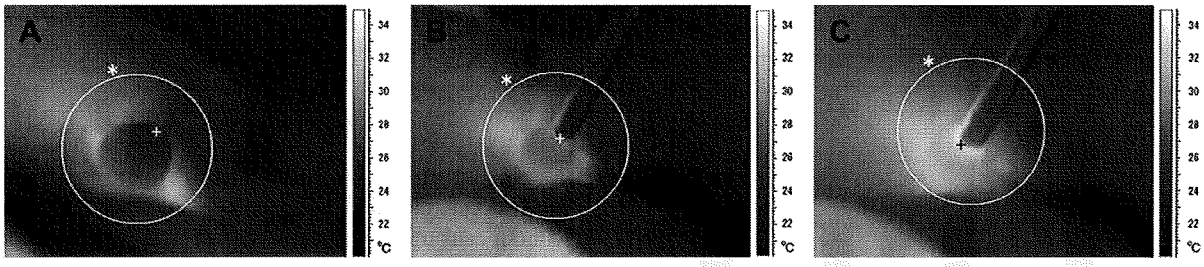


Fig. 5. The thermographic images of ultrasound-treated eye by an infrared thermography. Temperature was expressed as a pseudo-color. (A) Control. The highest temperature within the circle (denoted by an asterisk) was 31.9°C and the temperature of the spot + was 24.4°C. (B) US exposure for 5 min under the condition: frequency of 1.0 MHz, duty cycle of 5.2%, pulse repetition frequency of 20 Hz and $I_{SPPA.3}$ of 0.228 W/cm^2. The highest temperature within the circle (denoted by an asterisk) was 31.2°C and the temperature of the spot + was 28.5°C. (C) US exposure for 5 min under the condition: frequency of 1.0 MHz, duty cycle of 100%, continuous wave mode and $I_{SPPA.3}$ of 0.156 W/cm^2. The highest temperature within the circle (denoted by an asterisk) was the area of the spot +, which was 33.7°C.

tubes and that might have augmented ultrasound effect excessively. The unexpected reflection might have been involved in this phenomenon. However, it was unlikely that the present fibrinolysis was caused by thermal effect, because there was no change in temperature in the tube before and after ultrasound in this in vitro system even by the prolonged exposure (20 min). Importantly, the goal of our study is clinical application of US for intraocular fibrinolysis, which could be achieved in rat eyes. It requires further studies to elucidate the real mechanism of the present phenomenon.

Heating is a concern for tissue damage but it could accelerate clot-lysis on the other hand. Francis et al. (1992, 1995) reported that US exposure is associated with only a minimal increase of clot temperature even at 4 W/cm^2, which would be more potent than our conditions. In this study, the clot temperature *in vitro* shows less increase under the condition: frequency of 1.0 MHz, duty cycle of 5.2%, pulse repetition frequency of 20 Hz and $I_{SPPA.3}$of 10.123 W/cm^2 and the ocular surface temperature *in vivo* showed a minimal increase under the condition: frequency of 1.0 MHz, duty cycle of 5.2%, pulse repetition frequency of 20 Hz and $I_{SPPA.3}$ of 0.228 W/cm^2. Thus, it is likely that a nonthermal mechanism played a central role in our observations. Given the results of the *in vitro* study, it is understandable that intracameral fibrinolysis was accelerated by US exposure in rats. However, *in vivo* conditions are totally different from those *in vitro*.

In our previous study using the same model, there were various pro- or antifibrinolytic materials in the anterior chamber such as tPA and its inhibitor, unlike in vitro experiments (Sakamoto et al. 1999). Additionally, the intraocular environment is not stable and is strongly modulated by other factors. For example, if severe inflammation occurred, intracameral fibrin was easily formed while intracameral fibrin disappeared after the inflammation had gone. On the other hand, platelets were reported to be activated *in vitro* by US exposure (Chater and Williams 1977). Thus, it was of note that US exposure under the present condition caused fibrinolysis in the present study.

Under normal conditions, anterior chamber fluid is transparent and tPA is dominant over plasminogen

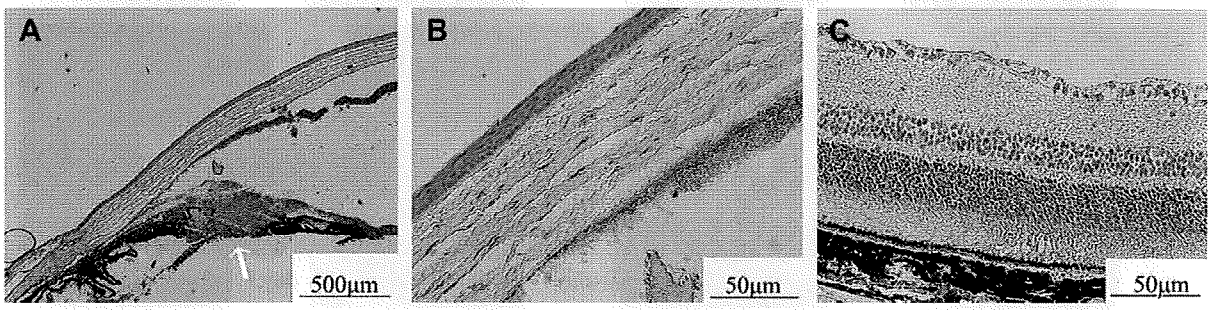


Fig. 6. Histologic photographs of rat eyes after YAG laser shot, before US exposure. (A) The only limited area of iris (arrow) was destroyed, called iridectomy hole, and 1/3 of the anterior chamber was filled with fibrin clot and red blood cells. (B) Fibrin clot and fibrin deposit were also found just beneath the corneal endothelium. There was not any apparent damage in other ocular tissues including cornea (B) and retina (C). Hematoxylin and eosin staining. Original magnification is (A) ×4, (B) ×40 and (C) ×40.

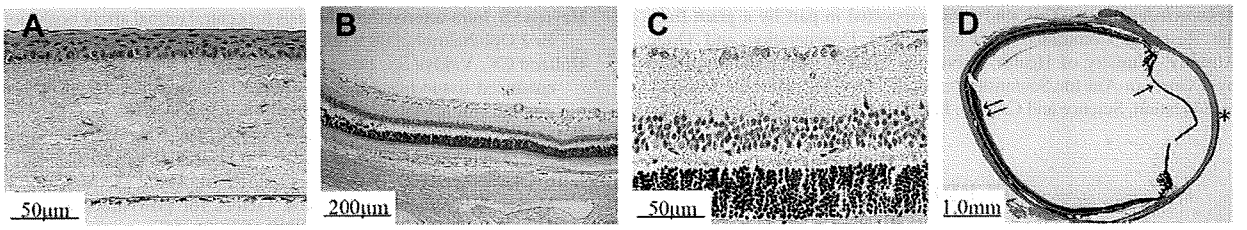


Fig. 7. Histologic photographs of rat eyes after US exposure. Original structure is well preserved and neither degeneration nor inflammation is found in cornea (A), retino-choroid (B) or retina (C). (D) The eyeball of the rat. Asterisk indicates cornea, arrow indicates iris and double arrow indicates retina. Hematoxylin and eosin staining. Original magnification is (A) ×40, (B) ×4, (C) ×40 and (D) ×2.

activator inhibitor (Fukushima et al. 1989). Fibrinolytic materials such as tPA were also assumed to be dominant over inhibitors after iris bleeding in this model because intracameral fibrin decreased gradually without any treatment. Therefore, US treatment accelerated intracameral fibrinolysis associated with tPA to some extent. Further study is necessary to clarify the mechanism in more detail.

Unlike the *in vitro* study, the additional injection of tPA to US exposure did not augment intracameral fibrinolysis. In this study, we did not use a direct intraocular injection of tPA because the intraocular injection itself could influence the intraocular fibrinolytic system. Instead, tPA was injected into subconjunctiva to avoid this destabilizing factor. As a result, it could not further enhance fibrinolysis. It was possible that subconjunctival tPA was degraded or did not penetrate the anterior chamber and thus active tPA might not be present sufficiently in the anterior chamber. An improved drug delivery system would be necessary.

US is a routine diagnostic procedure for ocular diseases and therapeutic application to glaucoma and intraocular tumor has already reported (Coleman et al. 1985, 1988). US is also believed to be a promising therapeutic alternative by several experimental studies (Sonoda et al. 2006; Yamashita et al. 2007; White et al. 2008; Zderic et al. 2004). However, US has still not been accepted for clinical use in most of ocular diseases including intraocular hemorrhage and vascular disorders.

This is not only because therapeutic value has not been well developed but there have also been concerns about its potentially harmful effects (Brown 1984). For example, the corneal endothelium *in vitro* was damaged by US exposure (Saito et al. 1999).

As this study reveals, the effects of US were influenced by various factors. Among them, the duty ratio was the strongest factor inducing a possible harmful event. In our *in vivo* study, the surface temperature of rat eyes increased to about 34 °C with the following conditions: frequency of 1.0 MHz and duty cycle of 100%, which was higher than periocular area while the surface temperature was not changed so much with a duty cycle of 5.2%. It is difficult to conclude that the present treatment is not harmless; however, it should be noted that a beneficial effect, intracameral fibrinolysis, could be obtained without causing a harmful event, histologically or clinically. Obviously, a human eye is much bigger than a rat eye and careful setting of US conditions would enable the therapeutic value of this treatment to be established.

There are many ocular diseases to which this treatment is potentially applicable. Of them, RAO is a good candidate. As is often quoted, a disease without any treatment has many treatments and RAO is a good example. The onset-to-treatment interval is the most critical issue for the successful treatment of RAO because longer periods of tissue ischemia result in irreversible retinal damage and permanent dysfunction. In a study with primates, the interval should be less than a few hours (Hayreh 2008; Hayreh et al. 2004). In comparison to surgery or intraocular injection, the present treatment is less invasive and does not need specific preparations for treatment (*e.g.*, disinfection treatment), which might waste precious time for effective treatment. Furthermore, US exposure might also be effective for removing cholesterol or calcium emboli because US can induce mechanical vibration. Considering these benefits, the present treatment should be worthy of study in a future clinical setting, although there are still many issues to be solved.

It is of note that our animal model is not that of retinal artery occlusion. To our knowledge, there is no reproducible animal model of retinal artery occlusion that is suitable for evaluating therapies. In contrast, the present model is suitable for studying the effect of intervention on intraocular fibrinolysis *in vivo*. This would give us the important information to develop a new treatment of retinal artery occlusion.

In conclusion, the present study shows that US exposure from outside can accelerate intraocular fibrinolysis. A beneficial effect was obtained without causing apparent damage. The US power was within the safety range of FDA regulations. Therefore, there might be more suitable ocular conditions for US treatment than given in the examples above. The present results could provide basic evidence to justify US treatment for ocular diseases related to fibrin formation.