Review

Novel Therapeutic Applications of Ultrasound. Utilization of Thermal and Cavitational Effects

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Abstract : Biophysical modes of action of ultrasound are classified as thermal and non-thermal effects. Non-thermal effects of ultrasound include cavitational and non-cavitational effects. The thermal effects of ultrasound have already been used for hyperthermic cancer therapy as well as microwaves and radiofrequency heating. Recently among the non-thermal effects of ultrasound, acoustic cavitation is considered to have the highest potential for therapeutic applications. Many examples of therapeutic applications for which cavitation may be involved have been reported, including sonodynamic therapy, gene therapy, thrombolysis and the use of microbubbles for enhancing ultrasonic action. This subject is of interest because it is related to the development of novel therapeutic applications using ultrasound. The role of cavitational effects in the therapeutic use of ultrasound is reviewed.

Key Words : ultrasound, therapeutic application, sonodynamic therapy, gene therapy, thrombolysis

Introduction

Ultrasound is widely used for soft-tissue visualization in all branches of medicine. Recent advances in the technology of three dimensional ultrasonic imaging, tissue harmonic imaging and harmonic imaging combined with echo contrast agents are expected to result in further applications. Ultrasound is also widely employed for surgery, lithotripsy in urology, emulsification of cataracts in ophthalmology, physical therapy and hyperthermia for cancer therapy. Ultrasound has a low enough tissue attenuation coefficient to penetrate intervening tissues and deliver its energy to non-superficial objects and can be focused into small volumes. This is a unique advantage when compared to electromagnetic modalities such as laser beams or microwaves in the applications for noninvasive and selective treatment of nonsuperficial tumors. Use of ultrasonically induced thermal effects in such applications has been studied mainly in two categories:

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ultrasonic hyperthermia and high-intensity focused ultrasound (HIFU) surgery¹⁾. In the former approach, a tumor is heated preferentially above 43°C for more than a period of the order of 10 min. In the latter, the tumor tissue in a focal spot is heated above its coagulation temperature for a period in the order of a second. In comparison with the thermal effects, the therapeutic use of nonthermal effects such as cavitation effects, of ultrasound has been much less studied. Ultrasonically induced cavitation, so called acoustic cavitation, is known to be the primary cause of sonoluminescence, mechanical shock waves and sonochemical reactions producing reactive oxygen species^{2,3)}, and besides lithotripsy, may have the highest potential for therapeutic applications among the nonthermal effects of ultrasound provided it can be suitably controlled.

1. Overviews of therapeutic application of ultrasound. Focusing and enhancement.

Two different directions of therapeutic application of ultrasound, focusing and the use of microbubble agents and chemicals to enhance the effects of ultrasound, have been proposed (Table I).

First, concerning utilization of thermal effects, conventional ultrasonic hyperthermia for cancer therapy has succeeded to some extent⁴⁾. Recently, focused ultrasound has been used not only for cancer therapy but also for ablation therapy of benign prostatic hyperplasia⁵⁾. Site-specific control of gene expression, such as heat shock proteins, by focused ultrasound has been proposed because focusing increases the site specificity⁶⁾. In another application of the use of microbubble agents, enhancement of temperature increase in a phantom has been reported when ultrasonic hyperthermia was combined with a microbubble echo contrast agent, Albunex⁷⁾.

Second, concerning utilization of non-thermal cavitation effects, there have been two areas of development, the first involves focusing to improve the site-specificity and the second combinations with photosensitizers (see below as "sonodynamic" therapy) and other agents. Microbubble agents have been reported as enhancing substances of the biological as well as chemical effects of ultrasound⁸⁾.

2. Drug activation by ultrasound-"Sonodynamic" cancer therapy

Recently, *in vitro* and *in vivo* experiments have demonstrated that ultrasound can activate certain porphyrins such as hematoporphyrin and thereby induce significant antitumor effects. Suspended tumor cells were lysed by ultrasound at a much higher rate in the presence of such porphyrins than in their

Table I. Strategy of ultrasound for therapeutic applications

Non-focusing		Focusing
Utilization of thermal effects		
Conventional hyperthermia ⁴⁾	→	Tissue ablation (HIFU) ⁵⁾
↓		Site-specific control of gene-expression (e.g. hsp) ⁶⁾
Enhancement by microbubble agents ⁷⁾		
Utilization of non-thermal effects		
Drug activation (+ photosensitizer)	→	Site-specific therapy with various agents
(+ anti-cancer drug)		
↓		
(+ gene-carrier)		
(+ thrombolytic agent)		
Enhancement by microbubble agents ⁸⁾		

absence at concentrations for which the porphyrins by themselves show no cytotoxicity. Experimental tumors implanted into mice have been treated with ultrasound in combination with administration of such porphyrins and the tumor growth was significantly inhibited at an ultrasound intensity for which ultrasound alone showed no significant effect. From these experimental a new modality of tumor treatment, which is called "sonodynamic therapy", has been proposed by Umemura and his colleagues. Although hematoporphyrin or its derivatives were initially used as a "sonosensitizer" ⁹⁻¹³⁾, not only porphyrins ¹⁴⁻¹⁹⁾ including Ga-porphyrin complex ATX-70 ²⁰⁻²³⁾, but also other compounds, pheophorbide a ²⁴⁾, dimethylformamide ²⁵⁾, merocyanine ²⁶⁾, piroxicam ²⁷⁾, tenoxicam ²⁷⁾ and erythrosine ²⁸⁾, were reported as sonosensitizing agents (Table II).

The above described *in vitro* and *in vivo* experiments were performed under standing wave conditions because acoustic cavitation can more easily be induced reproducibly by standing waves than by progressive waves. In a standing wave field, microbubbles smaller than the resonant size are driven by the ultrasonic radiation force toward a pressure antinode, where they can efficiently grow and collapse. However, insonation with standing waves does not seem to be widely applicable to various therapeutic situations. In early studies on the biological effects of high-intensity focused ultrasound, in which a progressive wave field was employed, the tissue damage due to so-called inertial cavitation was only observed at ultrasonic intensities an order of magnitude larger than the intensity threshold for the thermal tissue damage. Some new method to efficiently generate acoustic cavitation even by progressive waves seems to be necessary to make sonodynamic treatment clinically useful. Microbubbles of acoustic cavitation in liquids or biological tissues are oscillators with such high nonlinearity that they radiate not only the integral harmonics but also the fractional harmonics of the insonated ultrasound. Therefore, it may

Table II. Sonodynamic therapy *in vitro* and *in vivo*

Chemicals	Ultrasound		Materials		Reference
	Frequency	Intensity	<i>in vitro</i>	<i>in vivo</i>	
Hematoporphyrin	1.9 MHz	~3.2 W/cm ²	Sarcoma 180 cells AH130 cells		9, 11)
	1.7 W/cm ²			Sarcoma 180/rat	10)
	10 W/cm ²		Rat erythrocytes		12)
(Photofrin II)	270 kHz	0.45 W/cm ²	HL-60 cells		13)
	210 kHz	1.3 W/cm ²		Liver tissue/rat	14)
	450 kHz	0.3 W/cm ²	Adult T-cell leukemia cells		15,16)
Porphyrins	1.9 MHz	~5 W/cm ²	Mouse leukemia L1210 cells		17)
	1 MHz	~18 W/cm ²	CHO cells		18)
Ga-porphyrin complex, ATX-70	1.9 MHz	~10 W/cm ²	Sarcoma 180 cells		19)
				Kidney tumor/mouse	20)
	50 kHz		HL-525 cells		21)
	0.5+1 MHz	~40 W/cm ²		Walker 256 carcinosarcome/rat	22)
Pheophorbide a	2 MHz	~3 W/cm ²	Sarcoma 180 cells		
		4.5 W/cm ²		Sarcoma 180/rat	23)
Dimethylformamide	985 KHz	~2.5 W/cm ²	HL-60 cells		24)
Merocyanine 540	255 KHz	0.4 W/cm ²	HL-60 cells		25)
Piroxicam, Tenoxicam	2 MHz	~3 W	Sarcoma 180 cells		26)
Erythrosine	0.5+1 MHz	~2 W/cm ²		Liver tissue/mouse	27)

be natural to expect that simultaneous insonation at harmonically related multiple frequencies might modify acoustic cavitation. Recently, simultaneous insonation of the fundamental and the second harmonics was utilized, effects of acoustic cavitation in a progressive wave field *in vitro* and *in vivo* was studied^{27, 28)}. Because it has been found that acoustic cavitation can be enhanced by an order of magnitude by superimposing the second harmonic on the fundamental. As a result, significant synergistic effects between the fundamental and the second harmonic on sonochemical reactions and on the threshold for inducing tissue damage were clearly demonstrated.

Initially, concerning the mechanism of "sonodynamic action" the involvement of singlet oxygen was suggested because cell damage enhanced by hematoporphyrin was suppressed by the addition of histidine but not by mannitol. Since SOD had some protective effect, it was considered that superoxide anion radicals also are involved in the enhancement¹¹⁾. In contrast, it has been reported that in the absence of sonodynamic sensitizers under conditions where standing waves are minimized and potential hyperthermic effects are eliminated, cytotoxicity is not linked to attack from free radicals formed outside the cells for Chinese hamster ovary (CHO) cells and immediate cell lysis is closely linked to the inertial cavitation, which is known to produce shear forces that disrupt cellular membranes¹⁸⁾. The experimental evidence suggests that sonosensitization is due to the chemical activation of sonosensitizers inside or in the close vicinity of hot collapsing cavitation bubbles to form sensitizer-derived free radicals either by direct pyrolysis or due to reaction with $\bullet\text{H}$ and $\bullet\text{OH}$ radicals, formed by pyrolysis of water. These free radicals (mostly carbon-centered) react with oxygen to form peroxy and alkoxy radicals. Unlike, $\bullet\text{OH}$ and $\bullet\text{H}$, which are also formed by pyrolysis inside cavitation bubbles, the reactivity of alkoxy and peroxy radicals with organic components dissolved in biological media is lower and hence have higher probability of reaching critical cellular sites²⁹⁾. Further investigations to clarify how sonosensitizers enhance ultrasound-induced cellular and/or tissue damages are required.

3. Application of ultrasound for gene therapy

Many procedures used for biologically, chemically or physically introducing DNA into cells have been successfully developed to date. They are varied in terms of efficiency, field of application, or complexity of the technique. Such procedures are being applied for gene therapy. However none meet all requirements for clinical use, such as high enough efficiency of gene introduction, correct delivery, combined with no genetic modification or adverse effects to the living organism, though a recent report of *in vivo* electroporation is encouraging³⁰⁾. Ultrasound can be focused to restrict the area of irradiation by monitoring with MRI^{6, 31)}. In addition it is now clear that ultrasound enhances efficient gene introduction *in vitro* and *in vivo*. It has thus become a viable candidate for gene therapy. We review here the present situation of the research on *in vitro* and *in vivo* transfection by ultrasound.

Ultrasound mediated transfection is a technique of relatively recent development initiated by Feccheimer *et al.*³²⁾, based on their technique that macromolecules could be introduced into the cytoplasm of living amoebae of the cellular slime mold, Dictyostelium discoideum, by ultrasound³³⁾. The next report by Kim *et al.* was published in 1996³⁴⁾. The substantial research on the transfection by the ultrasound commenced thereafter (Table III).

According to the series of reports, the transfection by ultrasound is a phenomenon that occurs when ultrasound is transmitted directly or through water or through the walls of a plastic culture dish to cells in

Table III. Ultrasound mediated gene transfer

Cell lines	Ultrasound	Comments	Efficiency	Reference
HeLa cells Hepatic tissue culture cells LTK- fibroblast Mouse lymphoma	Branson sonifier model 200, power set#1, 20 kHz	Comparison of scrape loading vs sonication loading Thymidine kinase gene Dependency of cell type	34 cells per 10 ⁶ living cells	32)
Rat primary fibroblast and chondrocyte	1 MHz, 0.3 MPa 0.4 MPa	C. W. + 20 or 30 sec pulse C. W. + 40 or 60 sec pulse No transfection at 3.5 MHz β -Gal positive or Neo R	2.4% to living cells	34)
Chinese hamster ovary (CHO) cells	2.25 MHz 0.2~0.8 MPa 60 rpm, Tube	Luciferase production 10% Albutex and rotation are essential. Cell phase independent	0.33 ng luciferase production per 10 ⁶ cells	37)
LnCap cells PC-3 cells	932 kHz, + burst repetition 10~10kHz 0.33 W/cm ² 20% D.F. ^{a)}	GFP plasmid+PI Flow cytometric analysis Transfection efficiency depends on tone burst frequency Dependency of cell type	20% to living cells	39)
HeLa cells C127 cells NIH/3T3 cells Human chondrocytes	1 MHz 0.5 W/cm ² 5 or 30 sec 1.0 MHz 0.12 MPa ~ 0.41 MPa +Albutex	Cationic liposome mediated transfection GFP reporter gene Enhancement by Albutex Reduction of total time by multiple short burst and Albutex	8 μ g/ml of CAT expression 50% to living cells	35) 40)
B16 mouse melanoma cells of rat <i>in vitro</i> and <i>in vivo</i>	Lithotripter Shock wave(SW) 24.4. MPa (+) 5.2 MPa (-) 100~800SW	Luciferase plasmid GFP plasmid Enhancement of <i>in vivo</i> transfection by air	1.4% (<i>in vivo</i>) 4.2% (<i>in vitro</i>)	43)
HeLa cells	Lithotripter SW ^{b)} 60~360 SW vs. focused US ^{c)} 1.18MHz 0.1~5 MPa	Luciferase plasmid Repetition frequency dependent transfection	0.8% (SW) 3% (US)	41)
Pocine vascular smooth muscle cells Endothelial cells	1 MHz, 0.4 W/cm ² 60s	Naked or liposome- complexed luciferase DNA	70 light unit/ μ g protein	36)

a) D.F.: duty factor

b) 13~19 kV, 19kV=69.5 MPa(+), 12.5 MPa (maximum peak)

c) US: ultrasound

isotonic solution containing DNA. A certain time after the irradiation gene expression can be observed. It was also shown that ultrasound irradiation facilitates transfection by cationic liposomes^{35, 36)}. Fluorescent labeled dextran^{32, 37, 38)} or propidium iodide tagged DNA molecules³⁹⁾ were used as a reporter instead of a gene in order to elucidate the mechanism of macromolecular uptake by ultrasound irradiation. These observations led to the hypothesis that the transfection by ultrasound may be mediated with pores that are transiently formed on the cell membrane. This process is accordingly called sonoporation in analogy with

electroporation.

It is considered that sonoporation is directly caused by inertial cavitation generated by ultrasound irradiation. Kim and colleagues observed gene introduction into cells by 1 MHz ultrasound irradiation which efficiently generated inertial cavitation whereas no transfection was seen with 3.5 MHz ultrasound that did not generate cavitation. They therefore concluded that inertial cavitation was related to gene introduction by the ultrasound³⁴⁾. It was also shown that providing cavitation nuclei by adding a contrast agent, Albunex, enhanced transfection efficiency by ultrasound^{37, 38, 40)} and that gene introduction was observed only when the ultrasound intensity was above the level that generates inertial cavitation (0.05 to 0.08 MPa) at 1 MHz³⁸⁾. From these observations it follows that inertial cavitation plays an important role in ultrasound mediated transfection. However, Huber and colleagues suggested in their report comparing ultrasound with the shock wave in transfection efficiency that other factors may also be involved in the mechanism⁴¹⁾. They showed that the efficiency of transfection was lower with shock waves than with ultrasound irradiation in spite of the higher efficiency of cavitation production with shock waves. The detailed mechanism in ultrasound transfection remains to be elucidated.

The concentration of DNA and the temperature are generally considered factors that may affect ultrasound transfection efficiency. In addition, the ultrasound frequency, intensity and the type of the wave (continuous or pulsed) need to be considered.

Regarding general factors, it can be expected that higher DNA concentrations and relatively higher temperatures would be better for transfection efficiency if one considers the mechanism in which generation of pores by ultrasound irradiation induces the exchange of substances between the cytoplasm and the cellular exterior fluid including a reporter DNA and the requirement for pores eventually to seal to contain the DNA inside. Higher concentrations accelerate the containment of DNA in the cell and higher temperatures may be enhancing the flexibility of the cellular membrane enhancing pore formation. Actually, when irradiated under different conditions, the transfection efficiency was linearly corresponded with the DNA concentration up to 80 $\mu\text{g/ml}$ ³⁴⁾ or 8 to 10 $\mu\text{g/ml}$ ⁴⁰⁾ and gene introduction observed at 37°C was not apparent at 23°C³⁴⁾.

As for the ultrasound frequency, it is related to the facility for generation of inertial cavitation in the liquid environment where gene introduction occurs. It is expected that a lower intensity is enough to achieve the transfection at a frequency for which ultrasound irradiation easily generates inertial cavitation. Therefore those two parameters should be considered reciprocally. At a low frequency inertial cavitation can be relatively easily produced. Actually gene introduction in the first report was achieved at 20 kHz, a low ultrasound frequency³²⁾. However since the produced cavitation bubbles are large relative to the size of the cells, their mechanical shear stresses are large enough to break cells down. With such mechanical shear stress it might be too difficult to adjust the intensity to control transfection efficiency. In the report by Fechheimer and colleagues³²⁾ the transfection efficiency was very low compared with those reported later with ultrasound at higher frequencies. The duration of irradiation they applied was only 0.5 seconds. On the other hand with ultrasound at higher frequencies, cavitation bubbles may be too small to generate enough shear stress to form pores on the cell membrane or it may be difficult to generate the cavitation with intensities that can be applied. In such cases the gene introduction may not be seen.

There are many studies on this subject. From the available data it appears that continuous wave ultrasound with a duration of 1 minutes at a frequency of 1.68 to 2.25 MHz and an intensity (mean peak

pressure amplitude) of 0.14 to 0.5 MPa is suitable for *in vitro* transfection^{37, 38)}. Ultrasound irradiation generating larger or more cavitation bubbles would result in a larger degree of cell lysis and a declining number of cells transfected. Conversely, smaller or less cavitation bubbles lead to less cell lysis but result in a lower transfection rate. Sonoporation is after all a compromise between damage and integrity of the cell membrane. Moreover, although the chemical effect of the ultrasound as represented by H_2O_2 generation suddenly starts to rise once the intensity passes the cavitation threshold, sonoporation follows a different course. It gradually appears as the intensity rises. This indicates that sonoporation is not directly involved in sonochemical processes and that probably mechanical processes associated with inertial cavitation are responsible³⁷⁾.

Concerning the type of the wave (continuous or pulsed), Tata and colleagues showed tone burst frequency dependent cell lysis and transfection in prostate cancer cell lines³⁹⁾. Although continuous wave irradiation resulted in low to medium efficiency of transfection, relatively low repetition cycle frequency irradiation at 10 to 150 Hz significantly enhances cell lysis and gene introduction by ultrasound at 932.7 kHz. A similar result with the ultrasound at 1.18 MHz was reported by another group⁴¹⁾. Kim *et al.* also observed a similar phenomenon. With the 1 MHz ultrasound at an intensity of 0.3 MPa it was similar to the results described above but continuous wave was the most effective when an intensity of 0.4 MPa was applied³⁴⁾.

Lithotripter shock waves can be also utilized for transfection. It was shown that its efficiency for the gene introduction is low to compared to focused sinusoidal ultrasound when applied *in vitro*⁴¹⁾. However lithotripter shock waves with a center frequency of 100 to 300 kHz have been reported to produce cavitation very effectively *in vivo*⁴²⁾. Actually Miller and colleagues reported that when lithotripter shock waves were used to irradiate tumor cells implanted in mice after injection of DNA and air, effective gene introduction into tumor cells occurred. They also observed a decrease in tumor volume with an increased number of shock waves, suggesting a novel cancer therapy by combining gene introduction and mechanical destruction of cancer tissue by shock waves⁴³⁾. It has also been reported that shock waves induce metastasis formation⁴⁴⁾, requiring further studies for future applications for cancer therapy.

As to *in vivo* transfection, there are two groups that have reported their research on this subject. One group reported a preliminary result about ultrasound irradiation of the rat knee joint after administration of *lacZ* gene³⁴⁾ and the other group reported introduction of the *GFP* gene into implanted tumor cells using lithotripter shock waves as described above^{43, 45)}. In both cases the ratio of gene introduction was promoted compared to corresponding controls without irradiation. Application of these techniques for clinical use is anticipated in the future.

It appears likely that from now on *in vivo* transfection will be the main subject of investigation. Although there are still many obstacles to be solved, research efforts have just started to develop a unique modality for gene therapy.

4. Application of ultrasound in thrombolysis

Standard treatment for patients with acute myocardial infarction (AMI) presenting early after onset of chest pain is systemic pharmacologic thrombolysis. However, reperfusion after the treatment is achieved in only approximately 50% of cases, and at least 6% of infarct arteries reocclude⁴⁶⁾. Routine adjunctive balloon angioplasty was shown to be ineffective in improving the results^{47, 48)}. Complications including

occlusive dissections, recurrent thrombus formation, and a high restenosis rate at follow-up represent the major factors limiting primary angioplasty. Accordingly, other technical approaches using laser⁴⁹⁾ or ultrasound energy have been suggested as alternative treatment methods.

Applications of ultrasound in thrombolysis are now divided into the following three directions see (Table IV)

(a) *Pharmacological thrombolysis supported by externally applied ultrasound*

In this field, many animal experiments have been carried out. Siegel *et al.* found that transcutaneous application of low-frequency (27 kHz) ultrasound over the chest significantly augmented t-PA-mediated coronary thrombolysis in a dog model of AMI⁵⁰⁾. Pathological examination confirmed the angiographic patency rate and did not reveal injury secondary to ultrasound in the skin, soft tissues, heart, or lungs. This is the first *in vivo* study to demonstrate that noninvasive ultrasound can be used to enhance coronary arterial thrombolysis in dogs. Data from this study and previous studies using similar transcutaneous, low-frequency ultrasound devices suggest that this method is safe and without acute adverse effects^{51,52)}.

Retinal vein occlusion, leading to loss of visual acuity, is the second most common vascular disease in the human eye. Thrombolytic treatment, with streptokinase and tissue plasminogen activator, is one of the most promising methods of treating a thrombus in the eye since it dissolves the clot, allowing the circulation to be re-established within a few hours. However, the thrombolytic treatment is not widely used today in retinal vein occlusion, because of severe side effects such as hemorrhage. If it were possible to lower the dose of the thrombolytic drug and thus lower the frequency of the side effects, and still maintain good thrombolytic effect by adding therapeutic US, it is probable that this treatment would be of greater

Table IV. Ultrasound thrombolysis in vitro and in vivo

Thrombolytic agent	Ultrasound (US)		Materials		Reference
	Frequency	Intensity	<i>in vitro</i>	<i>in vivo</i>	
(Transcutaneous application of US)					
t-PA	27 kHz	0.45 W/cm ² (anterior surface of the heart)		Dog AMI	50)
PESDA (microbubble)	91 kHz			Rabbit iliofemoral artery	52)
SK	26 kHz	18 W/cm ² (at the tip of the US transducer)		Rabbit iliofemoral artery	53)
SK	1 MHz	1 W/cm ² (at the tip of the US transducer)		Rabbit retinal vein	54)
(Catheter-delivered US)					
-	20 kHz			Dog femoral artery	56, 57)
-	19.5, 45 kHz			Human AMI	58-60)
-	41.9 kHz			Human SVGs	64)
(US at high frequency)					
t-PA, UK	0.5, 1 MHz	8 W/cm ²	Clot		65, 66)
t-PA, UK, SK	1, 2.2 MHz	4 or 8 W/cm ²	Clot		67)
SK	1 MHz	1 W/cm ²	Clot		69)
UK	1.3 MHz	0.3 W/cm ²	Clot		70)
UK+microbubble	170 kHz	0.5 W/cm ²	Clot		71)
t-PA	1 MHz	1.75 W/cm ²	Rabbit jugular vein		68)

UK; urokinase, SK; streptokinase, AMI; acute myocardial infarction, SVGs; saphenous vein grafts

clinical interest. Larsson *et al.* indicated that adding pulsed ultrasound of 1 MHz made it possible to lower the dose of streptokinase while maintaining a good thrombolytic effect⁵⁴⁾. They found significant enhancement of streptokinase by ultrasound in the rabbit retinal veins occlusion model.

(b) *Lysis of intravascular clots by intravascular ultrasound*

External ultrasound applications have some problems: (i) in external application, relatively high energy is needed at the surface of the body to sufficiently deliver ultrasound to deeply located thrombus, (ii) ultrasound must propagate long distances through tissues which could cause physical and chemical side effects, (iii) air pockets in the lung or intestines may prevent ultrasound energy from reaching the target thrombus, (iv) multiple transducers and the large size of the ultrasound equipment needed for extracorporeal exposure could elevate medical costs⁵⁵⁾. To overcome these problems, there are many studies concerning internal ultrasound application.

Catheter-delivered, therapeutic ultrasound (20 kHz) has been shown to dissolve thrombi effectively without ultrasound-related arterial wall injury in dogs^{56,57)}. In addition to animal experiments, clinical applications of intravascular ultrasound have been studied^{58-60, 64)}. Hamm and coworkers published a case report on a patient with AMI who was successfully treated with ultrasound (19.5 kHz)-assisted balloon angioplasty⁵⁹⁾. Rosenschein *et al.* evaluated the clinical feasibility of percutaneous transluminal coronary ultrasound (45 kHz) thrombolysis in AMI for the first time⁶⁰⁾. Ultrasound induced successful reperfusion in 87% of the patients. Final angiographic results revealed minimal residual stenosis (20%) with no adverse morphological signs on angiography. Ultrasound thrombolysis has the potential to be a safe and effective catheter-based therapeutic modality in reperfusion therapy for patients with AMI. The skills required to operate the coronary ultrasound thrombolysis system are similar to those required for coronary balloon angioplasty. The catheter was easily delivered to the target coronary arterial segment, and precise positioning or coaxiality of the catheter was not crucial.

Saphenous vein grafts (SVGs) occlusion is the predominant cause of recurrent ischemia in patients who have undergone coronary bypass surgery⁶¹⁾. The rate of occlusion in grafts at 5 years after operation is 45%⁶²⁾. The management of recurrent ischemia in patients who have occluded venous bypass grafts is challenging. Reoperation is associated with higher mortality and morbidity, whereas the likelihood of achieving complete relief of symptoms is lower than in the first surgery⁶³⁾. Rosenschein *et al.* also reported the first experience with US thrombolysis in thrombus-rich lesions in saphenous vein grafts (SVGs), most of which were occluded⁵⁴⁾. Procedural success against occluded SVGs by ultrasound (41.9 kHz) was obtained in 65% of the patients, with a final residual stenosis of $5 \pm 8\%$. There was a low rate of device-related adverse events. Ultrasound thrombolysis in thrombus-rich lesions in SVGs might offer a very promising therapeutic option.

(c) *Pharmacological thrombolysis accelerated by intravascular ultrasound*

Many clinical trials concerning applications of ultrasound at low frequencies (20 kHz) have been conducted as mentioned above. On the other hand, ultrasound at lower intensities and much higher frequencies has been used in order to enhance pharmacological thrombolysis. Francis *et al.* have shown that ultrasound at 0.5-2.2 MHz can accelerate thrombolysis mediated by t-PA *in vitro*⁶⁵⁻⁶⁷⁾. Tachibana showed that an echo-contrast agent (Albunex) induced further acceleration of pharmacological thrombolysis by ultrasonic energy (170 kHz)⁷¹⁾.

Ultrasound at low frequencies (20 kHz) delivered by catheter creates violent cavitation and local large amplitude stirring, producing clot fragments of variable size. This might result in frequent occurrence of distal embolization during this intervention. The combination of ultrasound at high frequencies (1 MHz) and plasminogen activators might be better in order to prevent the distal embolization because the thrombolytic drugs could dissolve thrombi efficiently without producing clot fragments of variable size. Recently, clinical trials in Europe have been reported using miniature ultrasonic transducers at the tip of catheters which can release thrombolytic drugs during ultrasonic irradiation⁵⁵⁾. This new therapeutic and internal application makes possible to apply ultrasound and thrombolytic drugs at a shorter distance, to minimize damage to surrounding normal tissues.

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超音波の新しい治療応用：熱作用から キャビテーション作用の利用へ

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要 旨：超音波の生体作用は熱作用と非熱作用に分類される。非熱作用はさらにキャビテーション作用と非キャビテーション作用に分けられる。超音波の熱作用はマイクロ波やラジオ波と同様にがん温熱療法に利用されてきた。近年、超音波の非熱作用のひとつであるキャビテーション作用を利用した治療応用の可能性を示す例が報告されるようになってきた。例えば“音響化学療法”，超音波を用いた遺伝子導入法，血栓溶解療法，微小気泡による超音波効果の増強等である。これらの方法のなかには新しい治療手段として確立する可能性のあるものもあり，その将来性が期待されている。今回，これらの研究例を紹介し，キャビテーションの役割と問題点について考察する。