EXPERIMANTAL APPLICATION OF PULSED Ho:YAG LASER-INDUCED CAVITATIONAL SHOCK WAVE GENERATOR AS A NOVEL DRUG DELIVERY SYSTEM

T. Ohki*, J. Sato**, A. Nakagawa**, M. Kanbe***, Y. Sato**, K. Kato**, T. Hirano**, H. Uenohara****, M. Sun*, T. Tominaga**, K. Takayama*****

**Interdisciplinary Shock Wave Research Laboratory, Institute of Fluid Science, Tohoku University, Sendai, Japan.
** Department of Neurosurgery, Tohoku University Graduate School of Medicine, Sendai, Japan.
*** Department of Internal Medicine, Senseki Hospital, Ishinomaki, Japan.
****Department of Neurosurgery, National Hospital Organization Sendai Medical Center, Sendai, Japan.
***** Tohoku University Biomedical Engineering Research Organization.

ohki@rainbow.ifs.tohoku.ac.jp

Abstract: Recently, shock waves in medicine have been extended to the treatment for more delicate, localized and deeper lesions. Therefore, it is now obligatory to develop a specialized shock wave generator that is suitable for those purposes with the ability to be developed by endoscope or catheter. And also, drug delivery system which is applied shock wave is considered one of the topics of medical application of shock wave.

We have developed an novel shock wave generator using pulsed Ho:YAG laser as an energy source. The present device is remarkable in that it is possible to expose the center of shock wave to target tissue in a localozed area. And then, we evaluated the phyisical properties of pulsed Ho:YAG laser-induced cavitational shock waves by the pressure measurement, and also as first step of this study, in order to estimate the capability of shock wave which generated by this generator as the drug delivery system, the enhancement of chemotherapeutic effects using GCIY cell line

Introduction

Shock waves have been described as a minimally invasive medical treatment modality, several million patients have been treated by the extracorporeal shock wave lithotripsy [1] all over the world, mainly for the fragmentation of renal and gallbladder stones [2] and for stones at other locations (bile duct [3] , pancreas [4] and salivary gland [5]).

In addition, attempts have been made to apply shock waves to long bones for bone formation and to pain centers for treatment of tennis elbow, painful heel, and calcifying tendonitis of the shoulder. And also, they have been reported to be useful for cardiac and cerebral emboli, for drug delivery system due to the abilities of shock waves which could enhance chemotherapeutic effects in vitro and in vivo experiments [6].

From the viewpoint of application of shock waves in neurosurgery, it can be considered that shock waves haves have abilities of treatment for enhancement of bone formation of cranial bone [7], treatment for nerve pain and effects as drug delivery for brain tumor, cerebral ischemia.

Previously, various methods have been used to genarete shock waves, witch are electromagnetic, electrohydraulic, piezoelectronic power and explosives of silver azide for clinical and experimental use. However, the sizes of these systems were relativery large for the neurosurgical use, and much attentions were expected in the treatment of more delicate, localized and deepper lesions for the neurosurgical use, especialy neuroendosopic surgery.

In respect of all these applications, the device of generating shock waves would have the following requirements, 1) it should be small size and light for the easiy and safe exposure of shock waves in the localized area, 2) it can be used with endoscope or catheters, 3) it does not resort to a shock wave focusing mechanism.

On the other hand, in our previous studies we investigated the generation of the liquid jet after irradiation by pulsed holmium:yttrium-alminum-garnet (Ho:YAG) laser within a capillary tube, and described it as the pulsed Ho:YAG laser-induced liquid jet [8].

Although in vitro experiments, it have demonstrated the possibility of applying this liquid jet to embolysis and to surgical dissection, we have focoused attention on the shock waves wich are generated by the collapse of the cloud of small cavitational bubbles that are induced when the pulsed Ho:YAG laser-induced bubbles in the narrow tube are pushed out of it in the water. as an extension of our previouos studies, we have developed novel shock wave generator in the hope that it will adress the above problems of previopuos shock wave generator.

In this study, the pulsed Ho:YAG laser-induced cavitational shock wave generator was made which can be inserted in to neuroendoscope. And then, we evaluated the phyisical properties of pulsed Ho:YAG laser-induced cavitational shock waves by the pressure measurement, and also as first step of this study, in

ISSN: 1727-1983

order to estimate the capability of shock wave which generated by this generator as the drug delivery system, the enhancement of chemotherapeutic effects using GCIY cell line [6].

Materials and Methods

1.The pulsed Ho:YAG laser-induced cavitational shock wave generator.

Fig.1 is the picture of the pulsed Ho:YAG laser-induced cavitational shock wave generator. The total weight of shock wave generator was 6.75 g. An underwater shock wave was generated by applying radiation with a pulsed Ho:YAG laser (Mid-infrared Pulse Laser System, Model MIPL-HQ, Sparkling Photon, Inc.; wavelength: 2.1µm, pulse dulation: 350 µs, maximum laser energy: 600 mJ, 3 shots/second). The laser was conveyed through an optical quartz fiber (core diameter: 600 µm). The distal end of a stainless steal tube (length: 220 mm, internal diameter: 1.0 mm, external diameter: 1.5 mm) was sealed with a Y-connector (ANPRUST®-C Y connector, AP-YC25S, Terumo Co., Ltd., Tokyo, Japan) to prevent air from emtering the system and supplied distilled water wich is supplied at a rate of 40 ml/hour by the syringe pump and cold at 5 ℃. The distance (L) between tip of optical quartz fiber and tip of stainless steel tube was fixed L = 4 mm. This generator can be inserted into the rigid neuroendoscope system (Hopkins Diagnostic Telescope, Codman. Co., Ltd., Tokyo, Japan).

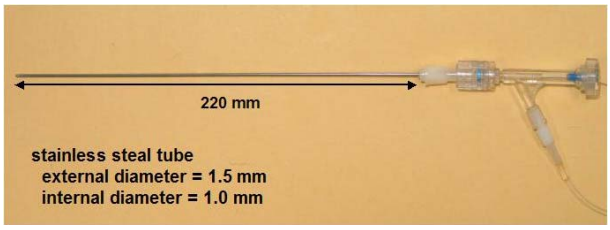

Figure 1: The pulsed Ho:YAG laser-induced shock wave generator.

2. Evaluation of physical properties of shock wave.

The underwater pressure profile of the laser-induced cavitational shock wave was eveluated by a PVDF needle hydrophone (serial No.300/25/230, Imotec Messtechnik, Wärendrof, Germany) with a 0.5 mm-sensing element of 0.0132 µV/Pa and a rise time of 45 ns, located D = 4-8 mm axially from the tip of the stainless steal tube (D: The distance between surface pf Latex membrane and tip of stainless steal tube) in the water chamber (Fig.2). The measured data were stored and displayed on a digital oscilloscope (model DL716; Yokogawa Co., Ltd., Tokyo, Japan). The cells exposed shock waves were placed in the Latex balloon, it is important to estimate the effect of Latex membrane on shock wave. There fore the relationship between the distance: D, overpressure and latex membrane was evaluated. The laser energy was fixed at 600 mJ/pulse.

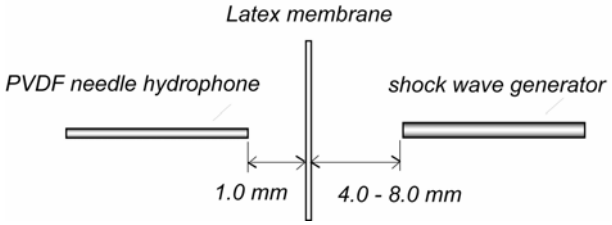

Figure 2: Experimental set up for pressure measurement.

3. Enhancement of Chemotherapeutic effects.

Experimental setting is shown in Fig.3. To generate shock wave, pulsed Ho:YAG laser-induced cavitational shock wave generator was used. GCIY gastric carcinoma cell line originary established from human malignant tumors was used, and cell suspention (1×10^6 /ml) were placed into rubber balloon made from Latex. the shock wave generator and balloons were placed in a water chamber equipped with holders for the shock wave generator and balloon, the distance between tip of shock wave generator and surface of balloon is fxed 4 mm. Shock wave exposure experiments in vitro were performed on 4 categories of cells, as follows :

A) non-treated.
B) treated with shock waves alone (10 Mpa: 2000, 4000, 6000 shots).
C) treated with BLM (anti-cancer drug) alone (0.01-100 µg/ml).
D) treated with combination of shock waves and BLM.

After these treatment, levels of cell proliferation were calculated by the MTT assay method.

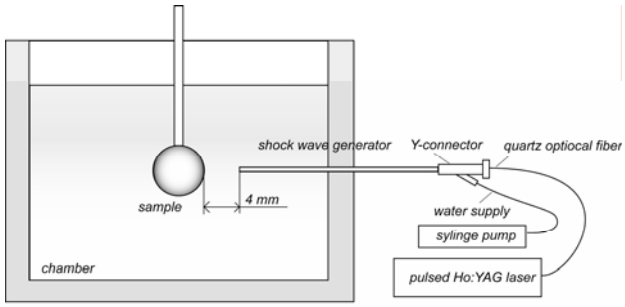

Figure 3: Experimental set up.

Results

Figure 4 shows the relationship between the distance from the tip of stainless steel tube and shock wave over pressure with Latex membrane or without it. The mean of over pressure at a distance of 4mm decreased from 19 to 10 MPa after placing Latex membrane.

ISSN: 1727-1983

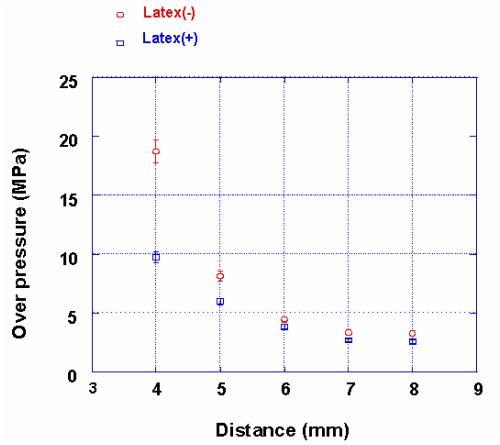

Figure 4: Results of pressure measurement.

The relationship between BLM concentration and % of cell proliferation is shown in Figure 5, the amounts of BLM are from 0.01 to 1000 µg/ml. When GCIY gastric cancer cells were treated with 4000 shots of shock waves at 10 MPa, 10-20 % of cells were damaged. Cell proliferation was markedly inhibited in proportion to the concentration of BLM. This inhibition was more evident when the cells were treated with 4000 shots than without shock waves exposure.

Figure 6 shows the relationship between BLM concentration and % of cell proliferation when the amount of BLM are 1-100 µg/ml and shock waves exposed 0, 2000, 4000, 6000 shots. Cell proliferation was markedly inhibited in times of shock wave exposure and proportion to the amount of BLM interactively.

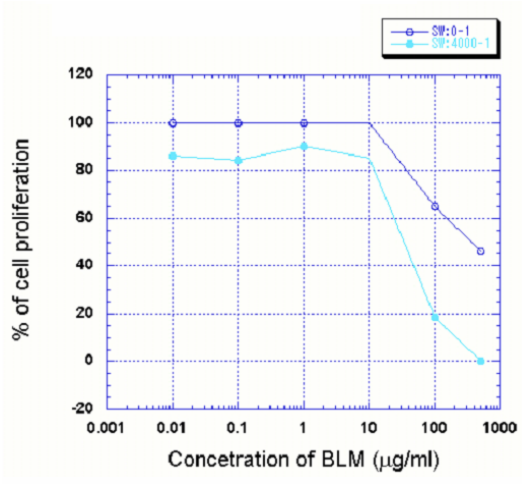

Figure 5: The relationship between BLM concentration and % of cell proliferation. (BLM: 0.1-1000 µg/ml, shock waves: 0, 4000 shots)

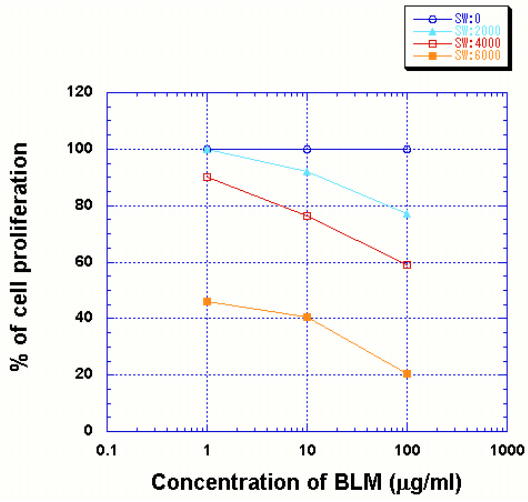

Figure 6: The relationship between BLM concentration and % of cell proliferation (BLM: 1-100 µg/ml, shock waves: 0, 2000, 4000, 6000 shots)

Discussion

Although clinical lithotriptors are the most common devices used to generate shock waves for clinical and experimental purpose, these devices require coupling with a relatively large area of the skin by using reflector in large aperture. In addition, a clinical lithotriptor requires a focusing system and has limitation with regard to the patient's position to avoid an acoustic impedance mismatch. Moreover, it is reported to be difficult to obtain focused, small-volume shock waves as for salivary gland stones that do not damage tissues outside the target, even when the desired focal point has been exactly determined by fluoroscopic and ultrasonic localization system. All of these problems made the application of shock waves in neurosurgical procedures difficult.

In contrast, the shock wave source in the current study is not only compact and light, but also can deliver the largest overpressure with minimal propagation beyond the target without the aid of a focusing system have been designed to gain overpressure of the generated shock wave effectively. Thus it is feasible to incorporate into catheters or endoscopes [9], making it suitable for delivering shock waves in cranial applications.

From the view point of enhancement of chemotherapeutic effects with shock waves, it has been reported that eukaryotic cells display a temporary increase in membrane permeability on exposure to shock waves, whose effect is caused by cavitations resulting in the transient generation of cell pores, allowing for the direct transfer of molecules of different sizes. Although the effects of shock waves on the blood-brain barrier and neuronal cells have yet to be investigated, we assert that one may achieve regional enhanced molecular transfer and drug delivery by the topical application of shock waves with the

ISSN: 1727-1983

simultaneous perfusion of molecules by using our method via a catheter or endoscopes.

As a next step of our study, the evaluation of anti-cancer cells and shock waves multiplier effects should be performed using brain cancer cells and other anti-cancer drugs.

Conclusions

These Results of the present study showed the possibilities of developing a method of shock wave generationg and novel drug delivery system in neurosurgery and other department that could not be use previouos system to generate shock waves.

References

[1] CHAUSSY C., BRENDAL W., SCHMIEDT E.(1980):'Extracorporeally induced destruction of kidney stones by shock waves',*Lancet*.,**2**,pp.1265-1268.

[2] SACKMANN M., PAULETZKI J., SAUERBRUCH T., et al.(1991):'The Munich Gallbladder Lithotripsy Study. Results of the first 5years with 711 patients', *Ann. Intern. Med*., **11**4, pp. 290-296.

[3] DELIUS M., GAMBIHLER S. (1992):'Sonographic imaging of extracorporeal shock wave effects in the liver and gallbladder wall of dogs', *Digestion*, **52**, pp. 55-60.

[4] SAUERBRUNCH T., HOLL J., SACKMANN M.,et al. (1987):'Disintegration of a pancreatic duct stone with extracorporeal shock waves in a patient with chronic pancreatitis', *Endoscopy*, **19**, pp. 207-208.

[5] BAYAR N., KAYMAZ FF., APAN A., et al. (2002):'Effects of electrohydraulic extracorporeal shock wave lithotripsy on submandibular gland in the rat: electron microscopic evaluation', *Int. J. Pediatr. Otorhinolaryngol*., **63**, pp.223-233.

[6] KAMBE M., IORITANI N., SHIRAI S.,et al. (1996):'Enhancement of chemotherapeutic effects with focused shock waves: Extracorporeal shock wave chemotherapy (ESWC)', *in vivo*.,**10**, pp.369-376.

[7] NAKAGAWA A., KUSAKA Y., HIRANO T., SAITO T., SHIRANE R., TAKAYAMA K., YOSHIMOTO T.,(2003):'Application of shock waves as a treatment modality in the vicinity of the brain and skull', *J. Neurosurg*., **99**, pp. 156-162.

[8] HIRANO T., KOMATSU M., SAEKI T., et al. :'Enhancement of fibrinolytics with a laser-induced liquid jet', *Lasers Surg. Med*., **29**, pp. 360-368.

[9] NAKAGAWA A., HIRANO T., KOMATSU M., et al.(2002):'Holmium YAG laser-induced liquid jet knife: possible novel method for dissection', *Lasers Surg. Med*.,**31**, pp. 129-135.

ISSN: 1727-1983