2P5-26

Proceedings of Symposium on Ultrasonic Electronics, Vol. 34 (2013) pp. 351-352
20-22 November, 2013

Antineoplastic effect of MHz ultrasound to leukemia cells

白血病細胞に対する MHz 超音波の抗腫瘍効果

Risa Fuji† and Wakako Hiraoka (Grad. of School. Sci. and Tech., Meiji Univ.)
藤里砂†，平岡和佳子 (明大院 理工)

1. Introduction

Recently, ultrasound has been a lot of attention in medical field. Its widely usage in diagnostic treatment fields such as bone density measurement and echocardiography, surgical treatment fields such as extracorporeal shock wave lithotripsy and high intensity focused ultrasound (HIFU) and drug therapy fields such as drug delivery system (DDS) has become an important issue.

In order to establish a safety and reliability in ultrasound (US) field especially in clinical therapy with ultrasound, it is necessary to clarify the physical and chemical effects of ultrasound and to elucidate the effect of physical and chemical mechanism on the tissue and organism. Recent report showed that the therapeutic level of ultrasound sometimes caused the dysfunction in normal cells and living tissue because of risk of free radical production such as hydroxyl radical (OH radical) in clinical US applications[1].

Ultrasound may cause important cell membrane modifications and irreversible cell damages. Several reports have suggested that cavitaion resulting from the collapse of gas bubbles generated by acoustic pressure fields may be the cause for cell damage following ultrasound irradiation[2-3]. Recently, in vitro studies demonstrated that therapeutic ultrasound induced apoptosis in cultured cells via the process of cavitation[4]. Apoptosis is programmed cell death to perform homeostasis exclusion of cell subjected to lethal damage, and it occurs in response to a large variety of signals including γ-irradiation and ultraviolet exposure. The apoptotic cells are digested by phagocytic cells such as macrophages to made clean and recycle. This is the utility of apoptosis for clinical treatment. Therefore in this study we used ultrasound at low energy to induce apoptosis specifically in leukemia cells and studied the biological effect of MHz ultrasound.

2. Materials and Methods

Experimental setup is shown in **Fig. 1**. For an ultrasound apparatus, ceramic resonators of 1.6 MHz and 2.4 MHz were used and placed in the bottom of water tank made of brass. A glass tube containing 2.0 ml cell suspension was set above the transducer on the water. The frequency of ultrasound was 1.6, 2.4, 5.4 and 7.9 MHz. The intensity of ultrasound was determined with calorimetric procedure. The test solution was irradiated for 10 seconds with continuous wave (CW) ultrasound. The colorimetric assay of liberated I_2 from KI solution was used for detecting OH radical. Human leukemia cells (U937) maintained in logarithmic growth phase were collected and resuspended at 1.0×10^6 cells/ml in 2.0 ml of RPMI-1640 containing 10% fetal bovine serum. The cell rupture was assayed immediately after irradiation. The reproductive and apoptotic cell death were assessed 12 or 24 hours after irradiation. The cell rupture and reproductive cell death were measured with cell counter (Coulter Counter Z1, Beckman Coulter). The induction of apoptosis was analyzed with flowcytometry (Cytomics™ FC500, Beckman Coulter) using phosphatidylserine externalization detected with annexin V fluorescein isothiocyanate (FITC) and propidium iodide (PI). Mannitol (0.25 M), histidine (0.02 M), ascorbic acid (0.01 M) and ethanol (1.0%) in phosphate buffer or RPMI-1640 were employed as OH radical scavengers. Stock solution of scavengers were prepared as a phisiological condition (pH 7.2).

3. Results and Discussion

Fig. 2 shows the comparison of the rate of cell rupture and the amount of OH radical exposed to 1.6 MHz ultrasound for 10 seconds. The number of intact cells was decreased immediately after US

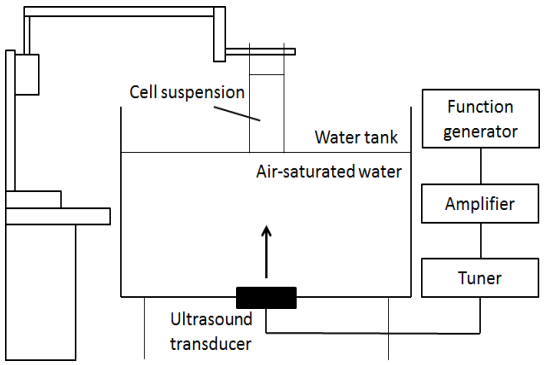

Fig. 1 Experimental setup.

hiraoka@isc.meiji.ac.jp

irradiation because the cells were ruptured during irradiation. OH-radical scavenger, 0.25 M mannitol, did not reduce cell rupture whereas OH radical was almost suppressed with mannitol. Data were shown almost the same as other types of OH radical scavengers such as 0.2 M histidine, 0.01 M ascorbic acid and 1.0% ethanol. Therefore, we concluded that cell rupture was caused by some mechanical effect of ultrasound other than the effect of cavitation-induced reactive oxygen species. From these results, ascorbic acid most efficiently showed OH suppression with the concentration of lowest cytotoxicity for 24 hours.

Fig. 3 shows the rate of cell proliferation 24 hours after irradiation of 1.6 MHz ultrasound for 10 seconds with or without 0.01 M ascorbic acid. The decrease of cell proliferation rate indicated the total cell damage, which contained rupture, dead, lysis and inhibition of growth. When the generation of OH radical is totally inhibited with 0.01 M ascorbic acid, the cell proliferation rate was increased compared than that of no scavenger.

Fig. 4 shows the data of flowcytometric analysis of apoptosis 24 hours after irradiation with OH scavenger. When cells were irradiated with 0.01 M ascorbic acid, flowcytometry indicated the increasing cluster of annexin V and PI-positive apoptotic cells and more intact morphology than that of authentic late apoptotic cells.

4. Conclusion

We examined the relationship between cellular damage and radical generation in CW-MHz ultrasound. Enough power to generate a large amount of OH radical produced excessive cell rupture during irradiation. When the OH radical was suppressed by scavengers, cell rupture was not affected. Furthermore, the decrease of OH by scavengers diminished a part of the reproductive cell death and induced apoptosis more efficiently. These results show that it is possible to suppress the proliferation of cells without causing excessive cell rupture. This ultrasound treatment could be a promising method for the elimination of leukemia cells by inducing apoptosis, and it may be a valid data for the establishment of the minimally invasive treatment.

References

1. GE. Kaufman *et al*: Ultrasound Med Biol. **3** (1977) 21.
2. JW. Ellwart, H. Brettel and LO. Kober: Ultrasound Med Biol. **14** (1988) 43.
3. M. Fahnestock *et al*: Ultrasound Med Biol. **15** (1989) 133.
4. H. Ashush *et al*: Cancer Res. **60** (2000) 1014.

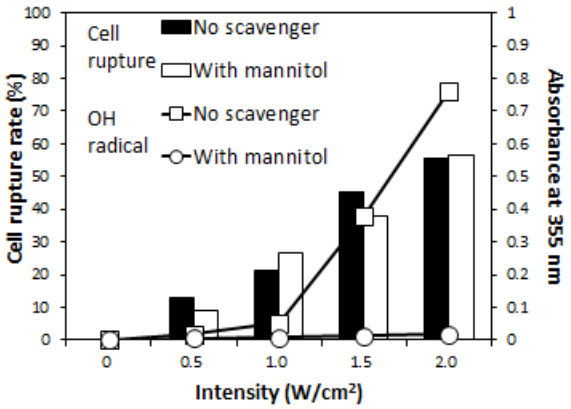

Fig. 2 Comparison of the rate of cell rupture and the amounts of OH radical with or without OH-radical scavenger (0.25 M mannitol). Bar charts and line graphs represent the cell rupture rate and the absorbance at 355 nm for OH radical generation, respectively.

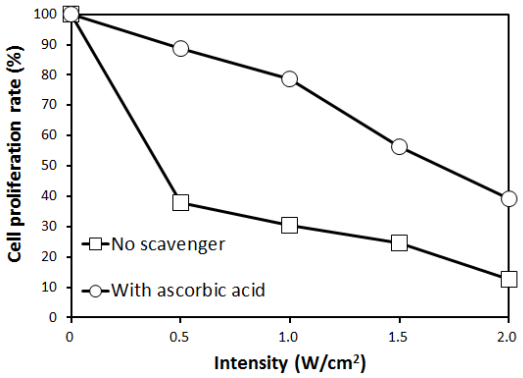

Fig. 3 Comparison of the rate of cell proliferation 24 hours after irradiation with or without OH radical scavenger (0.01 M ascorbic acid).

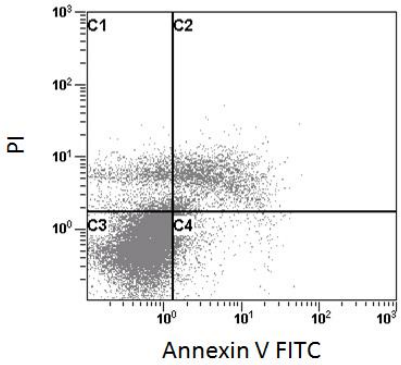

Fig. 4 Flowcytometric analysis of apoptosis 24 hours after irradiation with 0.01 M ascorbic acid. In each panel, the lower left, right, the upper right and left quadrants represent living cells, early apoptosis, late apoptosis and necrosis, respectively.