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Sonodynamically-Induced Cytotoxicity by Rose Bengal Derivative and Microbubbles in Isolated Sarcoma 180 Cells.

ローズベンガル誘導体とマイクロバブルによる腫瘍細胞に対する音響化学的細胞毒性

Nami Sugita[1†], Mami Hosokawa[2], Naoki Sunaga[2], Yumiko Iwase[2], Nagahiko Yumita[2], Toshihiko Ikeda[2], and Shin-ichiro Umemura[3] ([1]JBIC Res. Inst., JBIC; [2]Sch. of Pharm. Sci., Yokohama College of Pharm.; [3]Dep. of Biomed. Engr., Tohoku Univ.)
杉田 奈巳[1†], 細川 真美[2], 須永 尚毅[2], 岩瀬 由未子[2], 弓田 長彦[2], 池田 敏彦[2], 梅村 晋一郎[3] ([1]JBIC JBIC 研, [2]横薬大 薬物動態, [3]東北大院 工)

1. Introduction

Sonodynamic therapy (SDT) is a new and promising strategy for cancer treatment. It utilizes the synergistic effects of ultrasound (US) and chemicals (sonodynamic sensitizer: SDS)[1)]. Acoustic cavitation is the mechanism involved in sonochemical reaction[2)]. We have been investigating new SDS for efficient promotion of cavitation which are key to obtain sonodynamic effect[3), 4)]. We found rose bengal (RB) has high potential as SDS [2), 5)-8)]. In addition we synthesized a tumor accumulative derivative of RB (RBD) [9)] and clarified the RBD maintains the sonosensitizing ability of RB[10)]. In this study we examined the effect of microbubbles (MB) on sonodynamically induced cytotoxicity by RBD.

2. Materials and Methods

Tumor Cells: Sarcoma 180 cells were supplied by Meiji Seika Kaisha (Tokyo, Japan). The cells were passaged weekly through male ICR mice in the form of ascites.

Chemicals: As SDT, RBD were prepared by using the method reported in a previous paper[9)]. As MB, Sonazoid (SZ) was purchased from Daiichi-Sankyo (Tokyo, Japan). SZ is a one of the ultrasonic contrast agent. Dulbecco's phosphate-buffered saline (PBS, pH 7.4) and the other reagents were commercial products.

Insonation: The experimental set-up for the insonation is shown in **Fig.1**. The transducer and the lower part of a flat-bottomed glass container were submerged in degassed water in the container during 5, 15 or 30 s exposure at frequency of 1.92 MHz and an ultrasonic Intensity of 2.3 w/cm^2. Tumor cells were suspended in PBS (5x10^6 cells/ml) and exposed to US in standing wave mode with or without RBD and in the presence and absence of SZ.

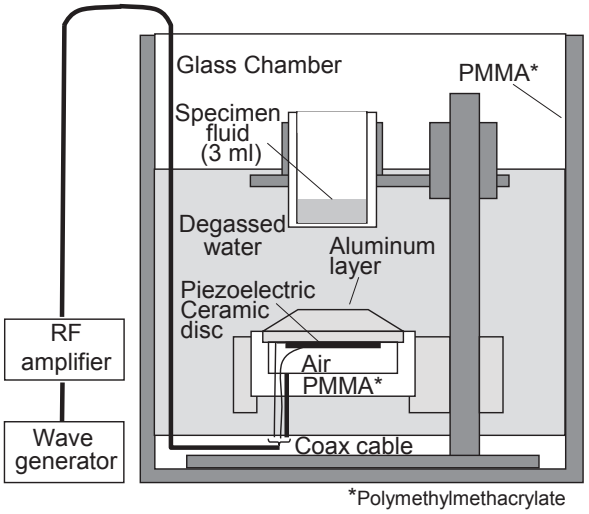

Fig. 1 Insonation apparatus set-up.

Evaluation of cytotoxicity: The viability of the isolated cells was determined by staining of the cells with trypan blue.

Evaluation of SZ distraction: The particle size of SZ was measured with submicron particle analyzer N5 (Beckman Coulter Inc.; Brea, USA).

3. Results and Discussions

Cytotoxicity: The unstained fractions of the isolated sarcoma 180 cells in the air-saturated suspensions, in the presence of 0 and 100 μM RBD and 0 and 200 μl SZ at an ultrasonic intensity 2.3 W/cm^2, are plotted versus exposure duration in **Fig.2**. When the cells were exposed to US alone or with RBD or with SZ, the unstained fractions plotted on a logarithmic scale decreased with exposure time primarily in a linear manner slightly. On the other hand, the cells were exposed to US in presence both RBD and SZ, the unstained fraction was reduced sharply to approximately 5% after the 5s duration. It is thought that SZ become the nucleus of the microparticle by cavitation and then

it let the ultrasonically induced cytotoxicity with RBD.

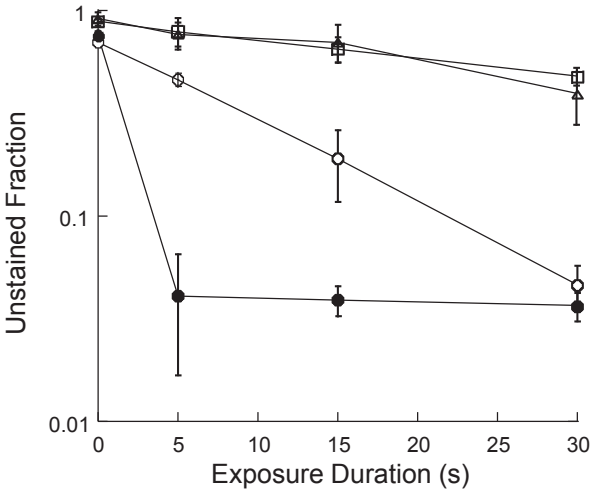

Fig. 2 Effect of RBD and/or SZ and US on isolated sarcoma 180 cells. Open squares are US alone, open triangles are US with SZ, open circles are US with RBD and solid circles are US with RBD and SZ. These are the mean values ± SD of three samples.

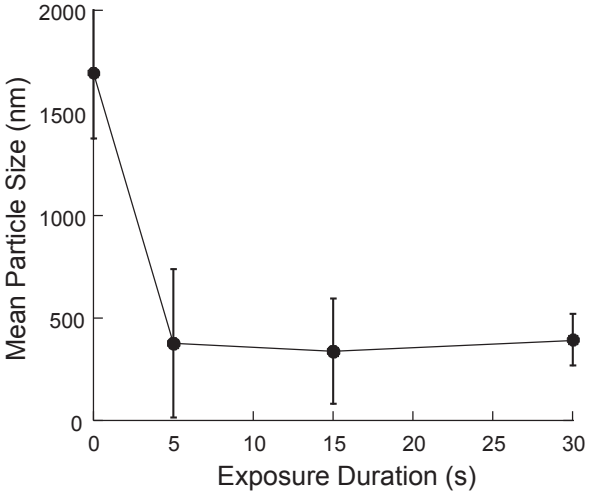

Fig. 3 Time course of SZ mean particle size during ultrasound exposure. These are the mean values ± SD of three samples.

SZ distraction: The SZ mean particle size in the air-saturated suspensions (10 ml SZ in 3 ml Milli Q water) after a fixed exposure time at an ultrasonic intensity 2.3 W/cm^2, are plotted versus duration in **Fig.3**. The SZ mean particle size was reduced to approximately 20% after the 5s duration.

The tendency of the collapse of SZ with US exposure accorded with that of the survival rate of the cell with US exposure in the presence of RBD and SZ. It was strongly suggested that the enhancement of sonodynamically induced cytotoxicity was caused by collapse of SZ.

4. Conclusions

The ultrasonically induced cytotoxicity with rose bengal derivatives and sonazoid was about 20 times higher than without them and about 80% of sonazoid was destructed, even for five-second exposure.

Since microbubbles induce to a significant cytotoxicity even for a short exposure duration and at a low intensity, these results suggest that the application of microbubbles for sonodynamic therapy is useful to achieve the efficient treatment. Microbubbles is presumed to play the role for promoting cavitation on this anti-tumor effect of sonodynamic therapy.

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Corresponding author email: sugita@jbic.or.jp