Immediate response and cytotoxicity effect on myocardial cells by extracellular photosensitization reaction varying irradiance

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Abstract— **We investigated the extracellular photosensitization effect on myocardial cells using talaporfin sodium at various irradiance measuring cell lethality dependence on talaporfin sodium concentration and immediate response of intracellular Ca^{2+} as a fundamental study of the application for tachyarrhythmia treatment. The myocardial cell lethality was measured 2 h after the photosensitization reaction by WST assay varying the irradiance from 0.12 to 0.66 W/cm^2. The intracellular Ca^{2+} of myocardial cell was observed during and until 10 min after the extracellular photosensitization reaction using fluo-4 AM and a confocal microscope varying the irradiance. The linear increasing behavior of the myocardial cell lethality with talaporfin sodium concentration was similarly obtained in the case of 0.12 and 0.29 W/cm^2 in irradiance. The photocytotoxicity was not obtained in the case of 0.46 and 0.66 W/cm^2 in irradiance. The time response of necrosis occurrence after the beginning of the extracellular photosensitization reaction was decreased with the irradiance increasing. We prospect that it may be caused by oxygen depletion in our *in vitro* system using 96 well plate since the irradiance and talaporfin sodium concentration were higher comparing the *in vitro* conditions of the general PDT for cancer.**

I. Introduction

The radio frequency catheter ablation is widely used as a definitive treatment for tachyarrhythmia [1]. The electrophysiological conduction block is made by Joul's heat generation by radio frequency current at the tip of the radio-frequency catheter to stop the abnormal impulse propagation that causes tachyarrhythmia. Severe thermal complications such as cardiac tamponade, pulmonary vein stenosis, cerebral infraction, phrenic nerve injury, and esophageal fistula are the problem [2]. We have proposed to apply the extracellular photosensitization reaction as a new non-thermal treatment methodology for tachyarrhythmia [3-6]. We intended to obtain the electrophysiological conduction block performance by oxidation of singlet oxygen produced by the extracellular photosensitization reaction with short drug-light interval. The immediate cell response is needed in the tachyarrhythmia treatment since the therapeutic effect and interventional operation completions are confirmed by the simultaneous electrophysiological diagnosis using mapping catheter during the operation. We think the immediate electrical conduction damage would be realized by ion channel or cell membrane damage and permanent electrical conduction damage would be realized by cell necrosis in the extracellular photosensitization reaction [3]. We have reported the cell killing effect by extracellular photosensitization reaction with various talaporfin sodium concentrations and radiant exposures [7]. The myocardial cell interaction with various irradiances is needed to understand the electrical conduction block myocardium that has certain depth since the irradiance decreased with the depth in myocardium by absorption and scattering. We studied the cell lethality effect and immediate cell response at various irradiances to understand the electrical conduction blockade effect in the myocardium.

II. Materials and Methods

A. Myocardial cell, Photosensitizer solution, Fluo-4 AM solution preparation

The primary rat myocardial cells (Primary Cell Company Ltd., Japan) was seeded in a black 96 well plate with 2.0×10^5 cells/ml in concentration for cell lethality measurement and in a 35 mmϕ culture dish with 4.0×10^5 cells/ml in concentration for intracellular Ca^{2+} measurement. The myocardial cells were cultured during 6-7 days for cell lethality measurement and 2-3 days for intracellular Ca^{2+} measurement in 37℃ and 5% CO_2. Dulbecco's modified Eagle medium/Nutrient Mixture F-12 (Invitrogen, USA) was used as culture medium and the medium was exchanged everyday.

Talapofin sodium (Meiji seika Pharma Co., Ltd., Japan) was used as photosensitizer. Talaporfin sodium was dissolved in the cultumedium described above under dark circumstance and the talaporfin sodium concentration was varied from 5 to 30 μg/ml.

Fluo-4 AM (Invitrogen, USA) of 50 μg was pre-dissolved in Dimethyl Sulphoxide (DEMSO; Invitrogen, USA) of 50 μl in volume. The fluo-4 AM solution of 7.5 μl in volume was diluted with Minimal Essential Media (Invitrogen, USA) of 1.5 ml in volume.

B. Cell lethality measurement

The prepared talaporfin sodium solution was injected in the well of the black 96 well plate. Every other well was used to not affect the neighboring well during the laser irradiation. The irradiances of 0.12, 0.29, 0.47, and 0.66 W/cm^2 were used. The radiant exposure was 20 J/cm^2.

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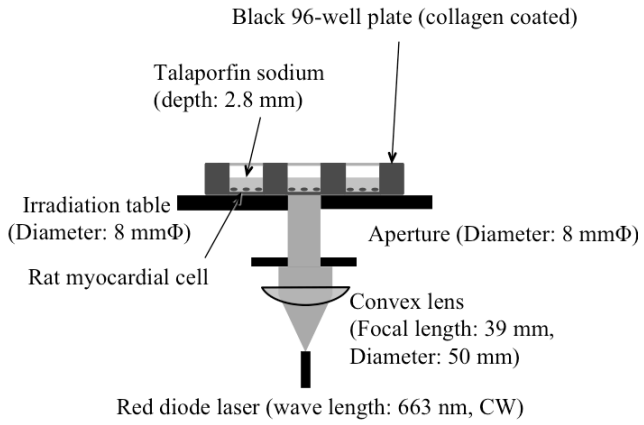

Figure 1. Experimental setup of the laser irradiation for the cell lethality measurement.

Fig. 1 shows the experimental setup for the laser irradiation. After the laser irradiation, water-soluble tetrazolium-8 assay solution (Cell Counting Kit-8; CCK-8, Doujinkagaku Company Ltd., Kumamoto, Japan) of 10 µl with the culture medium of 0.1 ml was injected in place of the photosensitizer solution. The laser irradiated myocardial cells were cultured with CCK-8 solution for 2 hours in the incubator. Absorbance at 450 nm of every well with laser irradiated myocardial cells in CCK-8 solution was measured by a microplate absorbance reader (Sunrise, Tecan Group Ltd., Switzerland). The absorbance at 450 nm in the well that has no damaged myocardial cells and CCK-8 solution without the photosensitization reaction was assumed as cell lethality of 0%. The absorbance of the well with completely damaged myocardial cells confirmed microscopic observation with CCK-8 solution was assumed as cell lethality of 100%. The cell lethality was calculated using these 2 references 2 hours after the photosensitization reaction.

C. *Intracellular Ca^{2+} measurement*

The prepared fluo-4 AM solution was contacted with the cultured myocardial cells in the 35 mmΦ culture dish during 20 min. A near infrared upright differential interference microscope (BX51WI-FL-IRDIC, Olympus, Japan) with a Nipkow type confocal unit (CSU-X1, Yokogawa, Japan) was used for the morphology and fluorescence observation. An Ar ion laser of 488 nm in wavelength (National laser, USA) was used for the excitation of fluo-4 AM connecting with CSU-X1. An electron multiplying CCD camera (DU897, Andor Technology, UK) was used for fluo-4 AM fluorescence observation. A near-infrared cooled CCD camera (Relora-XR, Qimaging, Canada) was used for the myocardial cell morphological observation. We constructed optical setup to irradiate the laser irradiation for extracellular photosensitization reaction under the microscope. Fig. 2 shows the optical system design of intracellular Ca^{2+} observation during the extracellular photosensitization reaction. A red diode laser of 663 nm in wavelength (Sony, Japan) was connected to the microscope to control the irradiation duration to vary the radiant exposure. The morphological and fluorescence observation and laser irradiation was performed through a water immersion objective lens (Olympus, Japan). The laser irradiation area was 5.1×10^{-3} mm^2 with the objective lens. The laser irradiation was regulated to 0.29 W/cm^2 measuring the laser power from the objective lens by a power meter (Coherent, Japan). The extracellular photosensitization reaction continuity was confirmed with this irradiance in previous study [8].

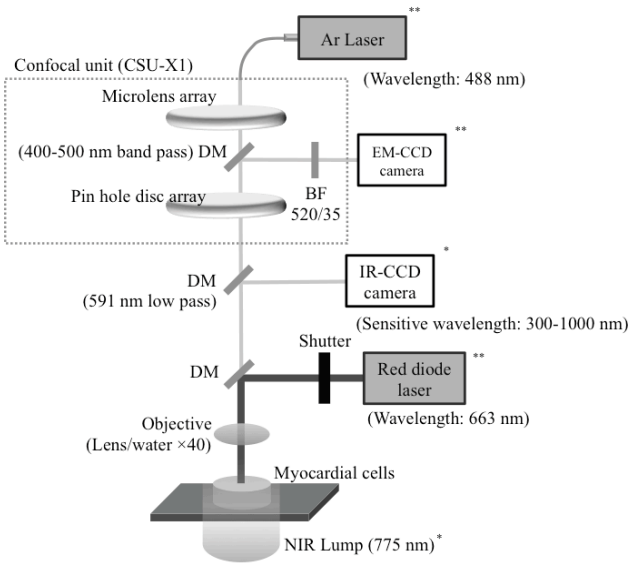

Figure 2. Optical system design of intracellular Ca^{2+} observation during the extracellular photosensitization reaction.

III. Results and Discussions

A. *Cell lethality dependence at various irradiances*

Fig. 3 shows the cell lethality dependence on talaporfin sodium concentration of different irradiance with radiant exposure of 20 J/cm^2. We found that the linear relation after certain talaporfin sodium concentration in the case of 0.12 and 0.29 W/cm^2 in irradiance and their dependences were similar. The talaporfin sodium concentration threshold was around 10-15 µg/ml in both cases. The photocytotoxicity was largely low in the case of 0.47 and 0.66 W/cm^2 in irradiance comparing the lower irradiances. The cell maximum cell lethality was less than 30% in these cases. We think that it is caused by oxygen depletion in our *in vitro* system using 96 well plate since the talaporfin sodium molecular number is much larger than the conventional *in vitro* system of photodynamic therapy for cancer. We found that the cell lethality has no dependence on the irradiance without the photosensitization reaction efficiency decrease caused by oxygen depletion of *in vitro* system and it increase with talaporfin sodium concentration increasing.

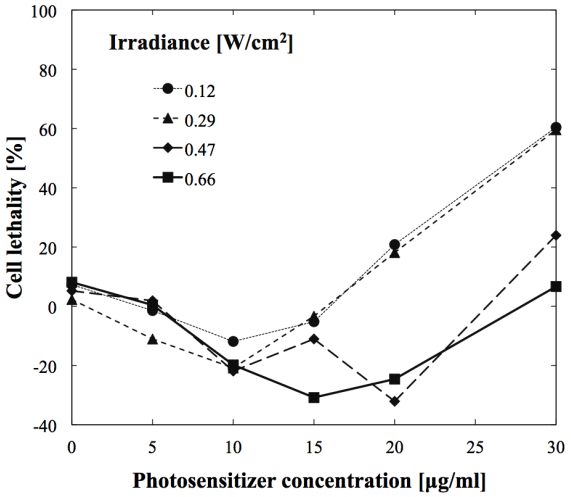

Figure 3. Cell lethality dependence of talaporfin sodium concentration at various irradiances. *(Radiant exposure: 20 J/cm², N=6)*

B. Immediate cell response at various irradiance

Fig. 4 and 5 shows the typical time response of normalized intracellular fluo-4 AM intensity during and after the extracellular photosensitization reaction with 0.12 and 0.29 W/cm^2 in irradiance. The laser was irradiated form 0 s to 136 s in the case of 0.29 W/cm^2 in irradiance and 0 s to 333 s in the case of 0.12 W/cm^2 in irradiance. The myocardial cells were contracted periodically before the laser irradiation and the intracellular Ca^{2+} was oscillated periodically. The intracellular Ca^{2+} oscillation was immediately stopped with the laser irradiation beginnings in the case of 0.29 W/cm^2. Intracellular Ca^{2+} was increased and cell necrosis was induced after that. The intracellular Ca^{2+} oscillation was stopped about 1 min after the laser irradiation beginnings in the case of 0.12 W/cm^2. Intracellular Ca^{2+} was increased and cell necrosis was induced after that similarly as 0.29 W/cm^2 in the irradiance. The time response to occur the myocardial cell necrosis was shorter in the case of 0.29 W/cm^2 in the irradiance. We found that we can control the time response to induce immediate cell necrosis occurrence varying irradiance but it not affect the cell lethality effect 2 hours after the extracellular photosensitization reaction.

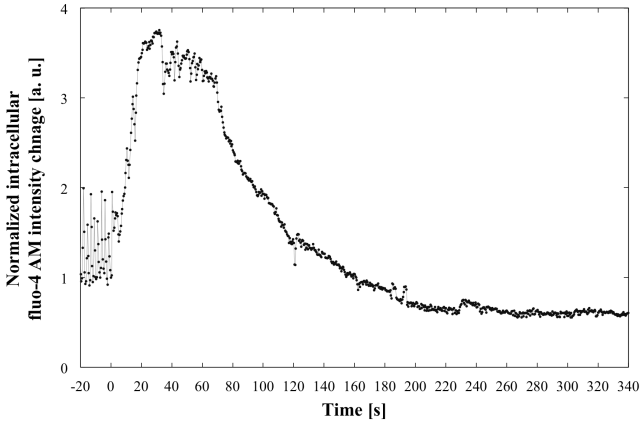

Figure 4. Typical time response of normalized intracellular fluo-4 AM intensity during and after the extracellular photosensitization reaction. *(Irradiance:0.29 W/cm²)*

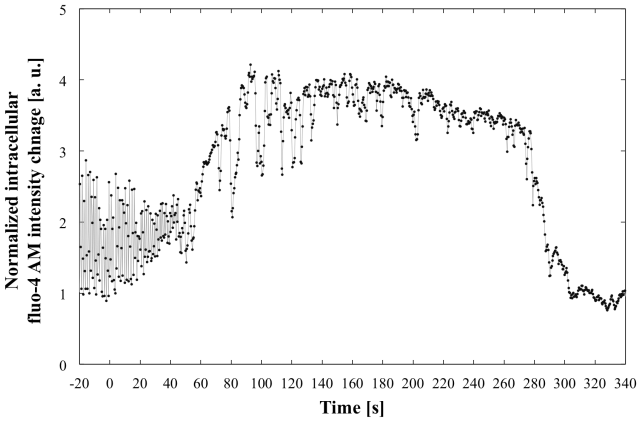

Figure 5. Typical time response of normalized intracellular fluo-4 AM intensity during and after the extracellular photosensitization reaction. *(Irradiance: 0.12 W/cm²)*

IV. CONCLUSION

We investigated the myocardial cell interaction by extracellular photosensitization reaction measuring cell lethality and immediate response of intracellular Ca^{2+} concentration. We found that the cell lethality 2 hours after the extracellular photosensitization reaction was not affect by the laser irradiance without the oxygen depletion of the in vitro system. We also found that we can control the time response to induce immediate cell necrosis occurrence varying irradiance. We think that the higher irradiance under the condition to prevent thermal effect is preferred in clinical application to shorter the ablation operation duration.

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