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Effects of albumin binding on photocytotoxicity of extracellular photosensitization reaction using talaporfin sodium to rat myocardial cells

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Summary
Background: We previously proposed a new treatment for tachyarrhythmia using an extracellular photosensitization reaction occurring in the interstitial space of myocardia shortly after the injection of talaporfin sodium. Using myocardial cells, we studied the photocytotoxicity of this extracellular photosensitization reaction between talaporfin sodium and albumin.
Methods: The albumin concentrations tested spanned the physiological range found in the interstitial space (0–15 mg/ml) while the talaporfin sodium concentration were varied from 0 to 40 μg/ml. The reactions were conducted in 96-well plates. To obtain the binding ratio and the amount of energy deposited into the photosensitizer, we measured the change in the absorbance spectra of talaporfin sodium solutions containing different concentrations of albumin.
Results: Photocytotoxicity to myocardial cell due to the reaction decreased when physiological concentrations of albumin were added to the reaction mix, and decreased sharply when the molar concentration ratio of albumin to talaporfin sodium was between 0.3 and 1.2. A monotonic binding ratio was obtained, ranging from 10 to 80%, at albumin concentrations of 0.1–1.0 mg/ml. We found that the lethality of the extracellular photosensitization reaction towards myocardial cells had a threshold albumin concentration, even though the energy deposited into the talaporfin sodium solution was calculated to be almost constant (4.23 ± 0.19 J/well) in the presence of 0–15 mg/ml albumin.

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Conclusions: Based on the likely concentration of albumin in the interstitial space, we conclude that the photodynamic efficacy of talaporfin, under conditions used here, will markedly decrease if the albumin level exceeds 0.65 mg/ml.

Introduction

Radiofrequency catheter ablation is widely used for atrial tachyarrhythmia treatment since it is generally superior to conventional rate and rhythm control drugs [1]. Radiofrequency catheter ablation causes myocardium electrical conduction blockage by thermal coagulation to shut down abnormal impulse propagations that cause tachyarrhythmia [2,3]. Severe complications caused by an increase in the temperature of the surrounding tissues are a problem, since temperature control inside tissues is difficult [4]. Radiofrequency catheters with irrigation cooling systems have been developed to prevent these complications [5]. However, these devices only reduce the surface temperature, restricting their ability to suppress complications. We have proposed a new, non-thermal treatment for tachyarrhythmia using talaporfin sodium in which an extracellular photosensitization reaction in the interstitial space of the myocardium is initiated by laser irradiation shortly after injection of the photosensitizer [6—9]. Talaporfin sodium is approved in Japan for treating early stage lung cancer and brain malignancies [10,11]. In contrast to cancer therapy, an immediate therapeutic effect is required in tachyarrhythmia treatment since electrical physiological analysis is used to judge the effectiveness of blocking myocardium electrical conduction during the ablation procedure. Permanent electrical conduction blockage is also required. The selective accumulation of photosensitizer in tumor tissues is utilized in cancer photodynamic therapy, with specified intervals between photosensitizer injection and laser irradiation [12]. Photosensitizer accumulation in tumor cells produces intracellular singlet oxygen that induces apoptosis [13]. The photosensitizer is not uptaken selectively by healthy tissues, such as myocardium, which we are targeting for tachyarrhythmia treatment. We therefore aimed to block the electrical conduction of myocardial tissue by an extracellular photosensitization reaction with laser irradiation *via* a laser catheter shortly after talaporfin sodium intravenous injection, since almost all talaporfin sodium is in the interstitial fluid and blood at that time [6]. We found that the extracellular photosensitization reaction can cause myocardial cell necrosis with Ca^{2+} inflow caused by membrane damage and ion channel dysfunction [7]. This cell damaging mechanism, which is different from the photodynamic therapy used for cancer, would provide an immediate response for tachyarrhythmia treatment. Talaporfin sodium is mainly bound to albumin *in vivo* [14]. In a cancer *in vitro* basic study of the intracellular photosensitization reaction, Sheyhedin *et al.* reported that cancer cell lethality changes according to the fetal calf serum (FCS) concentration in a solution containing talaporfin sodium. They also reported that the absorbance peak of the photosensitizer solution changed with the FCS concentration [15]. The cell killing effect in our proposed extracellular photosensitization reaction may be affected by the albumin concentration in the interstitial fluid even if the mechanism leading to cell death is completely different between the intracellular and extracellular photosensitization reaction. The albumin concentration may be as high as a few mg/ml in the interstitial space, where our intended reaction occurs, and therefore might affect the cytotoxicity of the photosensitization reaction. We investigated the effect of the binding between talaporfin sodium and albumin of the extracellular photosensitization reaction on cytotoxicity towards myocardial cells *in vitro*. The binding ratio between talaporfin sodium and albumin was obtained by absorbance spectroscopy from the shift in the talaporfin sodium Q band.

Materials and methods

Photosensitizer solution

Saline with bovine serum albumin (BSA) was used as a solvent for spectroscopic measurements. Dulbecco's modified Eagle's medium/nutrient mixture F-12 (Invitrogen, Carlsbad, CA, USA) with 10% fetal bovine serum (Invitrogen) was used as a culture medium and solvent for cell photocytotoxicity measurements. Talaporfin sodium (Laserphyrin®; molecular weight: 799.69; Meiji Seika Pharma Co., Ltd., Tokyo, Japan) was dissolved in each solvent in the dark. This photosensitizer is a hydrophilic chlorin family photosensitizer with high absorption in the Q band and quick egestion [10]. The absorption peak is at 664 nm and has a bandwidth of 20 nm [10].

Laser irradiation system

A continuous red diode laser with a wavelength of 663 ± 2 nm was used as an excitation light for the extracellular photosensitization reaction. The laser irradiance was applied homogenously in an 8-mm diameter hole on a black irradiation table to irradiate the well, from bottom to top, using the optical setup described in Fig. 1. Laser irradiance was set to 0.29 W/cm^2. This value was chosen from preliminary experiments designed to avoid oxygen depletion by high concentrations of the photosensitizer [16].

Photocytotoxicity measurement

Rat myocardial cells (Primary Cell Co., Ltd., Hokkaido, Japan) were cultured in collagen-coated black 96 microwell plates (Corning Inc., Corning, NY, USA) for 6—7 days in an incubator at 37 °C and 5% CO_2. Photosensitizer solution (0.1 ml) was added to each well. The solution depth was 2.8 mm. The photosensitizer concentration and albumin concentration were varied from 0—40 μg/ml to 0—15 mg/ml,