Study of photodynamic diagnosis for peritoneal metastasis using Talaporfin Sodium

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Abstract

The most common cause of death in Japan is cancer. Among them, the pancreatic cancer is difficult to detect at an early stage because there is a little subjective symptom at early stage, and it is often advanced cancer already when it was discovered. In addition, there is metastasis to the peritoneum as the destination of pancreatic cancer metastasis, which is called peritoneal metastatic cancer or peritoneal seeding. Since peritoneal dissemination is small in nodules, it does not clearly appear in CT or the like, and many conditions are very advanced even if it appears. In this study, we investigate the possibility of photodynamic diagnosis (PDD) of peritoneal dissemination using Talaporfin sodium, the second generation of photosensitizer.

Keyword: Talaporfin sodium, Photodynamic diagnosis, mouse model, peritoneal metastasis

Introduction

The most common cause of death in Japan is cancer. Among them, the pancreatic cancer is one of the 4th most common mortality per organ, and both mortality rate and morbidity rate are still rising. In addition, pancreatic cancer is difficult to detect at early stage because there is little subjective symptom, and it is become advanced cancer already when it was discovered. Also, as metastasis of pancreatic cancer, it is metastases to the peritoneum. Those in which cancer has metastasized from other organs to the peritoneum are called peritoneal metastatic cancer or peritoneal dissemination. Small nodules of small peritoneal seeding are small, so they can not show up clearly in CT and other diagnosis, and in many cases, they are ongoing at the time of discovery. As the treatment policy for organ cancer that becomes the primary cancer changes depending on the presence or absence of peritoneal dissemination. Early detection has the merit of improving the patient's quality of life (QOL). In this study, we investigate the possibility of photodynamic diagnosis (PDD) of peritoneal seeding using Talaporfin sodium, the second-generation photosensitive substance.

Material and Method

A peritoneal dissemination mouse model was prepared using MiaPaCa-2-GFP in which green fluorescent protein (GFP) was introduced into human pancreatic cancer cells (MiaPaCa-2). The photodynamic diagnosis was performed by a novel laparoscopic system. The cell MiaPaCa-2-GFP was cultured in a 100 mm dish in an incubator until reaching 70 - 80% confluence. Cells were detached from the dish using Trypsin EDTA and centrifuged. After stirring with fresh medium, the number of cells was measured, then HBSS physiological isotonic buffer salt solution was added and maintained on ice until administration to mice. A mouse model was prepared using the cultured cells. Mice were sleeping with ketamine + xylazine anesthesia and MiaPaCa-2-GFP (5.0×10^5 cells/mL) was injected intraperitoneally. Three weeks later, Talaporfin sodium was intravenously injected into the tail vein at 5 mg / kg. Sacrifice was performed 6 hours after administration, laparotomy was performed, and visible and infrared fluorescent images were obtained using a CCD camera. In addition, fluorescence spectra were measured using a fluorescence spectroscope. A laser of 664 nm (semiconductor laser, 30 mW) was used as an excitation wavelength of Talaporfin sodium. In addition, similar cell experiments using MiaPaCa-2-GFP were performed in order to investigate the results obtained with the mouse model. In the incubator, Talaporfin sodium adjusted to 1×10^{-6} M was taken in MiaPaCa-2-GFP cultured until it reached 70 to 80% confluency with a 100 mm dish, and then cells were collected from the dish using Trypsin EDTA , And transferred to a quartz cell having an optical path length of 1 mm to obtain a cell model. The visible image (VIS), infrared fluorescence image (IR), and fluorescence spectrum were measured using a 664 nm laser similarly to the mouse model.

Result and Discussion

The visible image (VIS) of the mouse model, the infrared fluorescence image (IR) and the fluorescence spectrum are shown in Fig.1.

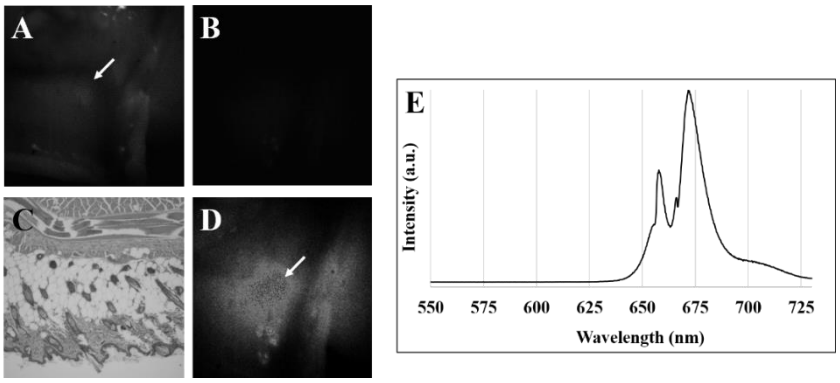

Fig.1 Fluorescence image and fluorescence spectrum of peritoneal dissemination mouse model with 664 nm laser excitation.

A. White light, B. 664nm laser – VIS, C. HE stain,

D. 664nm laser – IR, E. Fluorescence spectrum

When irradiated with white light, tumor-like nodules were not observed, but the fluorescence of GFP from the tumor site could be confirmed from visible image, so that the observation was made centered on that portion. When excited with a laser of 664 nm, the fluorescence of Talaporfin sodium could not be confirmed in the visible image, but the fluorescence of Talaporfin sodium was observed from the tumor site of the infrared fluorescence image. Similarly, when the fluorescence spectrum of the tumor site was measured, fluorescence of talaporfin sodium could be confirmed around 670 nm. The fluorescence of talaporfin sodium in the infrared fluorescence image was confirmed to coincide with the site where GFP fluorescence of the tumor confirmed by the naked eye appeared. As a result of pathological diagnosis by HE staining, cancer was diagnosed. Based on these results, we concluded that Talaporfin sodium can be used for peritoneal dissemination PDD.

The visible image (VIS), the infrared fluorescence image (IR) and the fluorescence spectrum of the cell model were measured and the results are shown in Fig.2..

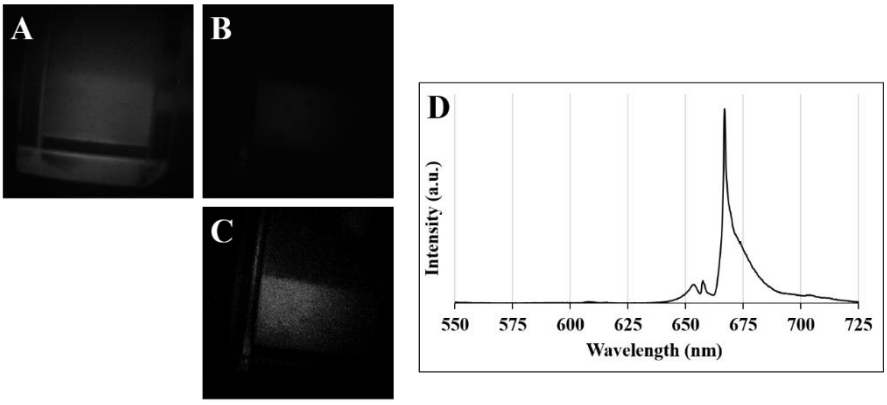

Fig 2. Fluorescence image of MiaPaCa-2-GFP by 664 nm laser excitation
And fluorescence spectrum
A. White light B. 664 nm laser - VIS
C. 664 nm laser - IR D. Fluorescence spectrum

When a 664 nm laser was irradiated to a quartz cell containing MiaPaCa-2-GFP, fluorescence could not be seen in a visible image, but fluorescence could be observed with an infrared fluorescence image. Also in the fluorescence spectrum, fluorescence of Talaporfin sodium is observed around 670 nm, so it can be confirmed that the same result as the result in the mouse experiment is obtained. Based on these findings, we concluded that Talaporfin sodium can be used for peritoneal dissemination PDD.

Future works

From this study, we confirmed the possibility of PDD of peritoneal dissemination using Talaporfin sodium. As a future policy, we will conduct similar duplication experiments and improve accuracy. We also intend to examine whether quantitative analysis is possible by carrying out cell experiments and mouse experiments using cell types related to peritoneal dissemination.

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